USER MANUAL

DAQ-PAC-F1S

1. List of parts

- **DAQ-1 instrument**
  - AutoResp™ 1
  - Power Cord
  - USB cable
  - Adapter cable for pumps, qty. 2
  - User manual

- **Fibox 3 or Microx TX3 fiber optic oxygen instrument**
  - Pt100 probe
  - Power Adapter
  - RS232 cable
  - User manual
  - PC operating software for Windows 98/00/ME/NT/XP

- **Input connectors, qty. 2**

- **Device connectors, qty. 2**

2. Contents

3. Installation procedure for DAQ-PAC-F1S

3.1 General

- AutoResp™ 1 is a piece of PC software designed to perform automated intermittent flow respirometry together with the DAQ-1 instrument.
- AutoResp™ 1 requires Windows XP running on a PC with an Intel Pentium IV processor of minimum 2.66 GHz or equivalent. The PC must have at least 256 MB memory and at least 1GB of free hard disk space. A minimum resolution of 1024x768 pixels is required.
- AutoResp™ 1 communicates with the DAQ-1 instrument via the accompanying USB cable which must be connected.
- AutoResp™ 1 can handle two different types of oxygen inputs. Either 0-5 VDC analog input (OXY-CH) or serial inputs when using fiber optic oxygen instrument manufactured by PreSens.
- If chamber oxygen is measured using the fiber optic oxygen instrument one RS232 male DB9 connector must be available on the PC. If not available use an USB 232 adapter, e.g. USB Serial Adapter manufactured by VSCOM.
- If using the fiber optic oxygen instrument turn this on before starting AutoResp™ 1.

Input labels, see Table 1.

Table 1

DAQ-1 instrument label	AutoResp™ 1 signal name
OXY-CH	Chamber oxygen
OXY-AM	Ambient oxygen
TEMP	Temperature
MOTOR	Motor

Output labels, see Table 2.

Table 2

DAQ-1 instrument label	AutoResp™ 1 indicator name
FLUSH	Flush
RECIRC	Recirc.
O2/N2	Ambient O_2 (kPa)
TEMP	Temperature (°C)

3.2 DAQ-1 instrument

3.2.1. Front side

OXY-CH: Analog oxygen input for chamber. The input range is 0-5 VDC.

Connect analog chamber oxygen input, e.g. from an OXY-REG, via pin 1 (+) and pin 4 (GND) in the input connector.

OXY-AM: Analog oxygen input for ambient water. The input range is 0-5 VDC.

Connect analog ambient oxygen input, e.g. from an OXY-REG, via pin 1 (+) and pin 4 (GND) in the input connector.

TEMP: Analog temperature input for ambient water. The input range is 0-5 VDC.

Connect analog temperature input, e.g. from a TMP-REG, via pin 1 (+) and pin 4 (GND) in the input connector.

MOTOR: Analog motor control instrument input. The input range is 0-5 VDC.

Connect analog motor input, e.g. motor controller instrument following with swim tunnel from Loligo Systems ApS, via pin 1 (+) and pin 4 (GND) in the input connector.

PC: USB port for PC communication. Use the USB cable to connect the DAQ-1 instrument to the PC.

LEDS: Relay status LEDs.

<u>IMPORTANT:</u> DO NOT connect DAQ-1 instrument to PC before installation of driver from Measurement & Automation software from National Instruments (see **Fejl! Henvisningskilde ikke fundet.**, page **Fejl! Bogmærke er ikke defineret.**) is completed. The software will be installed together with the installation of AutoResp™ 1, see page 6.

3.2.2. Back side

FLUSH: Relay socket for 110/230VAC (max 3A) flush pump.

Connect pump by using one of the accompanying adapter cables.

RECIRC: Relay socket for 110/230VAC (max 3A) recirculation pump.

Connect pump by using one of the accompanying adapter cables.

O2/N2: Relay socket for 110/230VAC (max 3A) for solenoid valve. Use oxygen or nitrogen for hyperoxic or hypoxic control.

Connect e.g. a solenoid valve, by using one of the accompanying device connectors.

TEMP: Relay socket for 110/230VAC (max 3A) heating/cooling.

Connect e.g. a heating/cooling, by using one of the accompanying device connectors.

100-240VAC 50-60 Hz: Power input for the DAQ-4 instrument.

Connect the instrument to a grounded 110/230VAC power supply using a standard pc-type cable with a grounded wall plug.

Power button: Turns the instrument on and off.

IMPORTANT: DO NOT connect any of the relay sockets to >3 amps equipment! USE grounded outlets only!

For further information about the DAQ-1 instrument see 8.3 DAQ-1 Instruction manual, page 50.

3.3 AutoResp™ 1 software for Windows

The following steps will explain how to install AutoResp™ 1 and drivers on your computer.

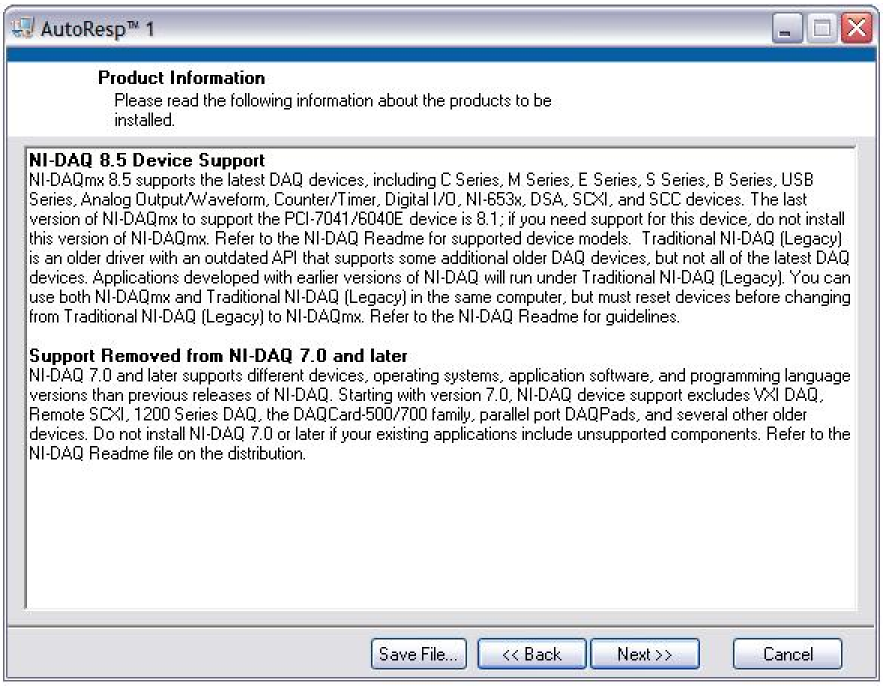
1. Insert the CD labelled AutoResp™ 1 and wait until you see Screen 1. If you do NOT se the screen, browse to the root of your CD and double click on the icon labelled setup.exe.

Screen 1

2. Click Next.

On Screen 2 the product information of the products to be installed are shown.

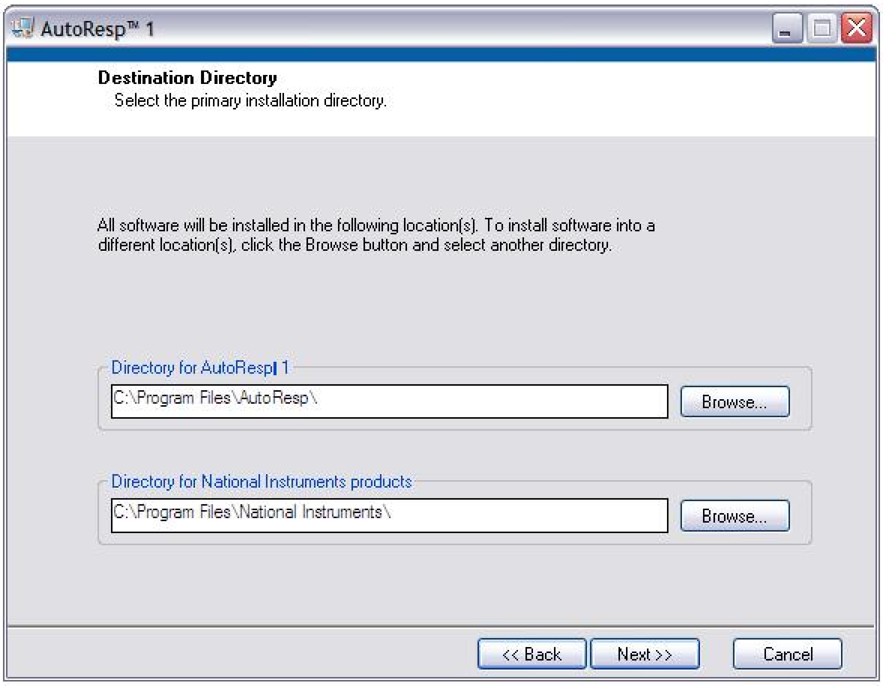
Screen 2



3. Click Next.

4. Select destination directory for AutoResp™ 1 and for the National Instruments driver.

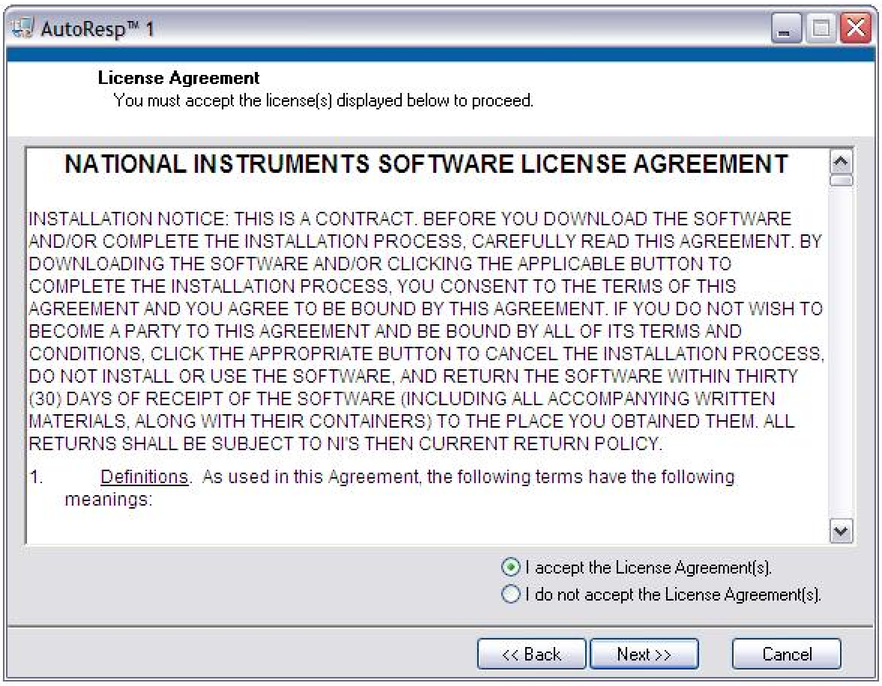
Screen 3



5. Click Next.

6. If you accept the License Agreement, please select “I accept the License Agreement(s).

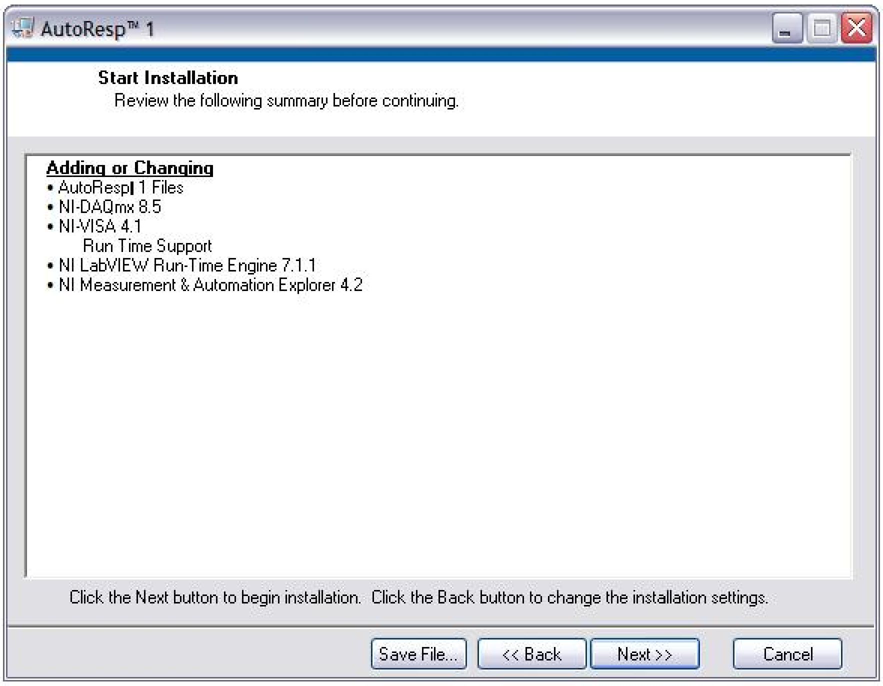
Screen 4



7. Then click Next.

On Screen 5 a summary is given of the products to be installed.

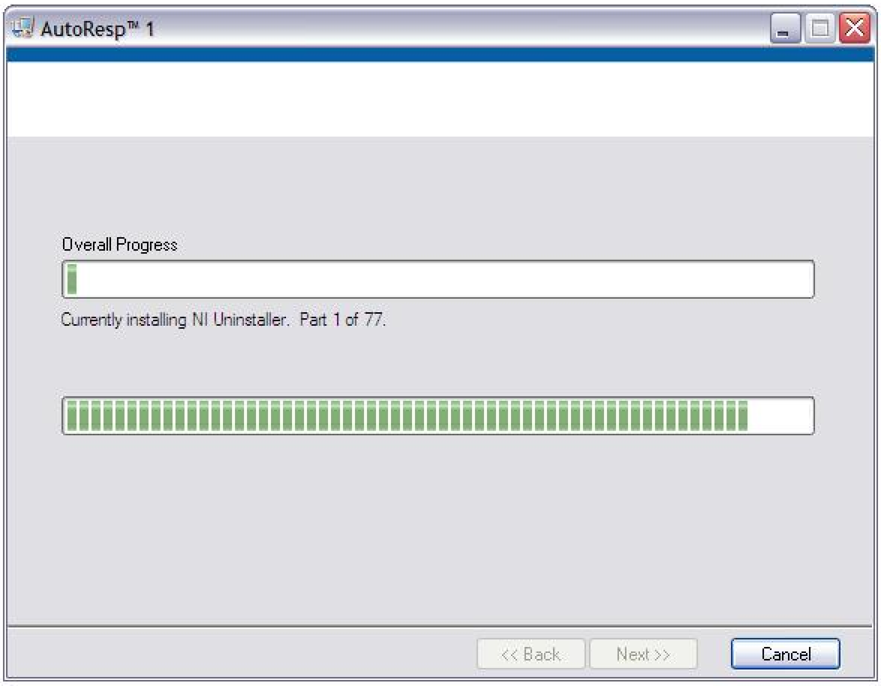
Screen 5



8. Click Next.

On Screen 6 the status of the installation is shown.

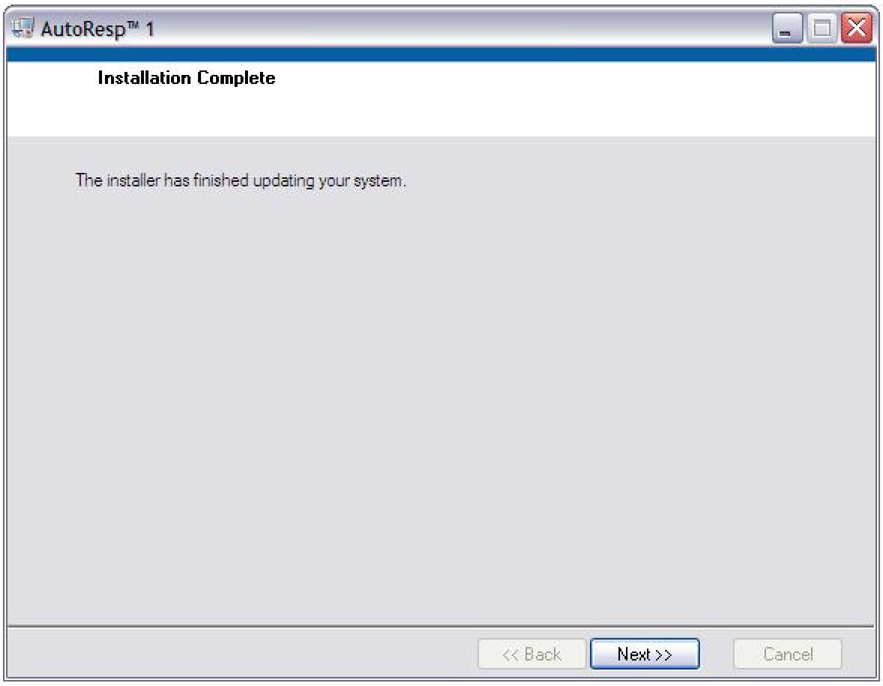
Screen 6



The installation may take a while.

When installation is complete Screen 7 becomes present.

Screen 7



9. Click Next to end installation.

10. If the USB hardware protection dongle drivers were not installed yet on the system then wait until you see Screen 8.

Screen 8

11. Restart the computer, but leave CD in the PC.

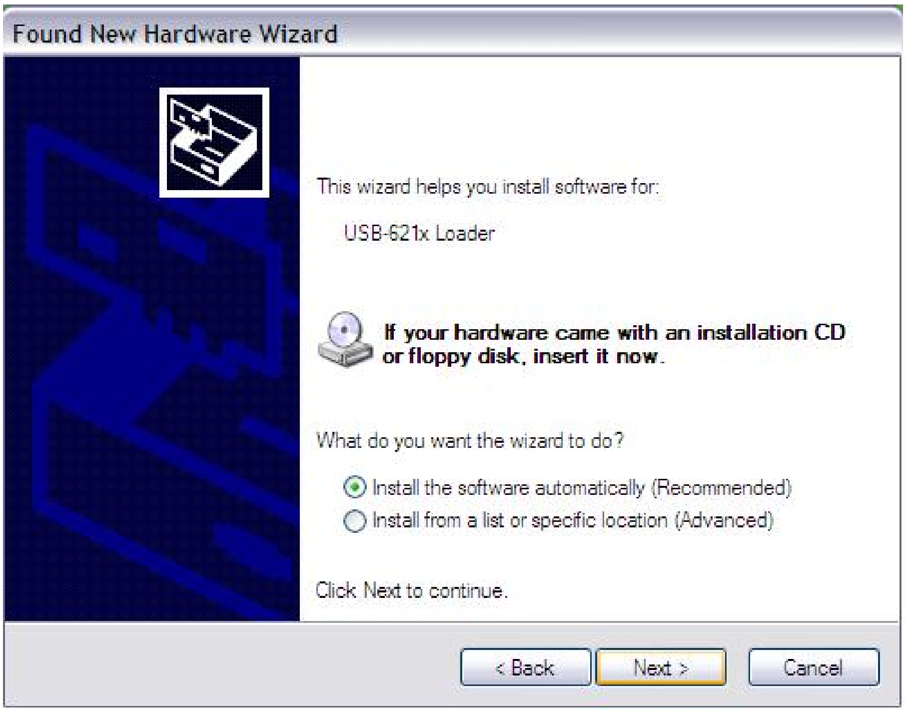
The following steps will explain how to install the DAQ-1 driver on your computer.

12. Connect the DAQ-1 instrument to the PC via the USB cable. After a few seconds Screen 9 becomes present.

Screen 9

13. Click on "No, not this time" and then on Next.

Screen 10



14. Click on "Install the software automatically (Recommended)" and then on Next.

Screen 11

15. Click Finish.

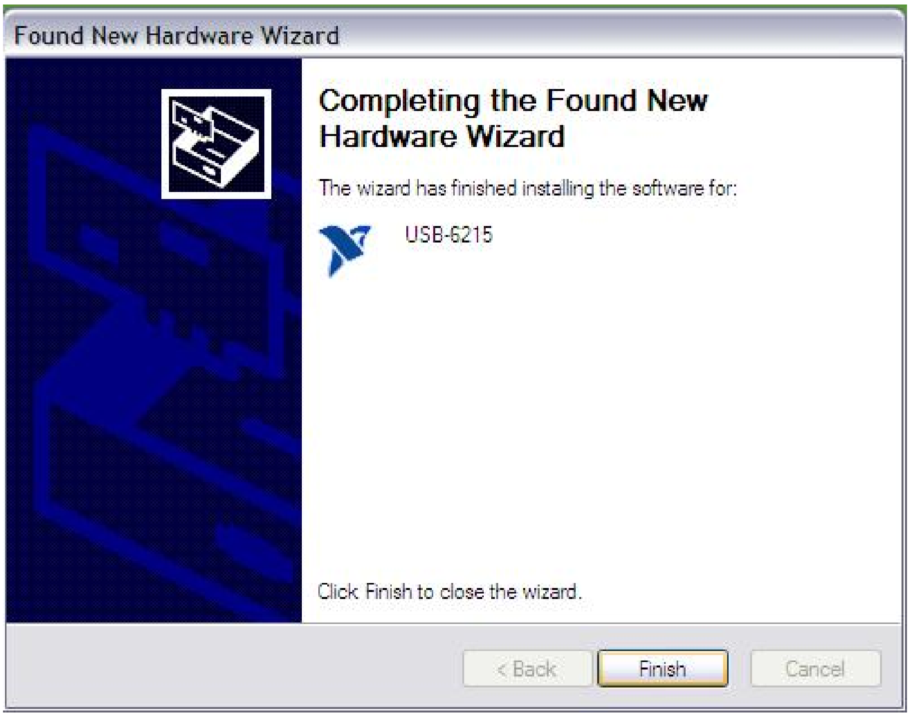
Screen 12

16. Click on “No, not this time” and then on Next.

Screen 13

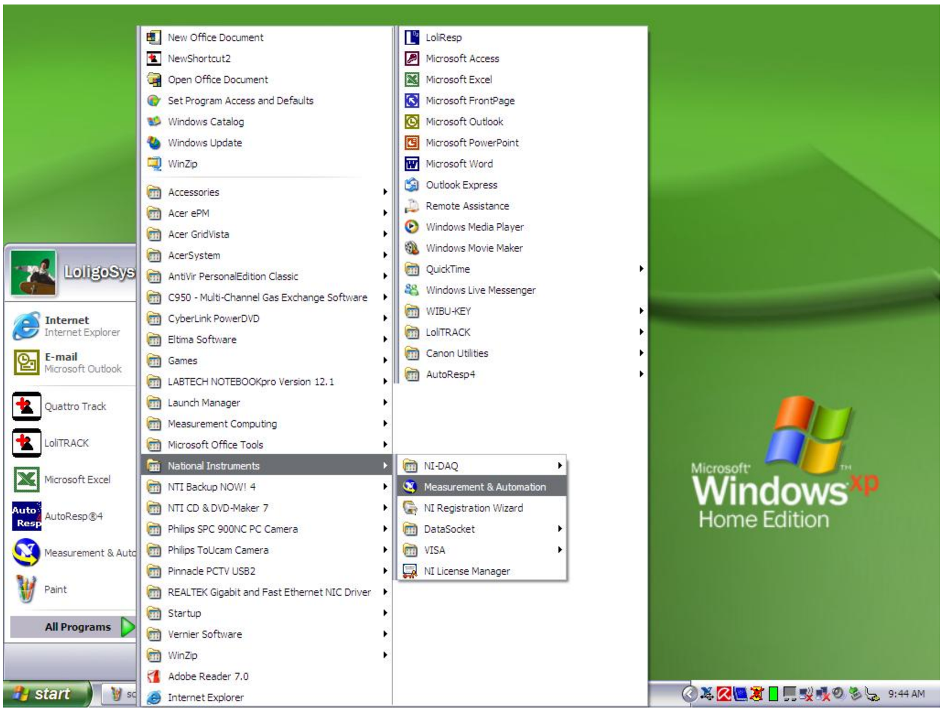
17. Click on “Install the software automatically (Recommended)” and then on Next.

Screen 14



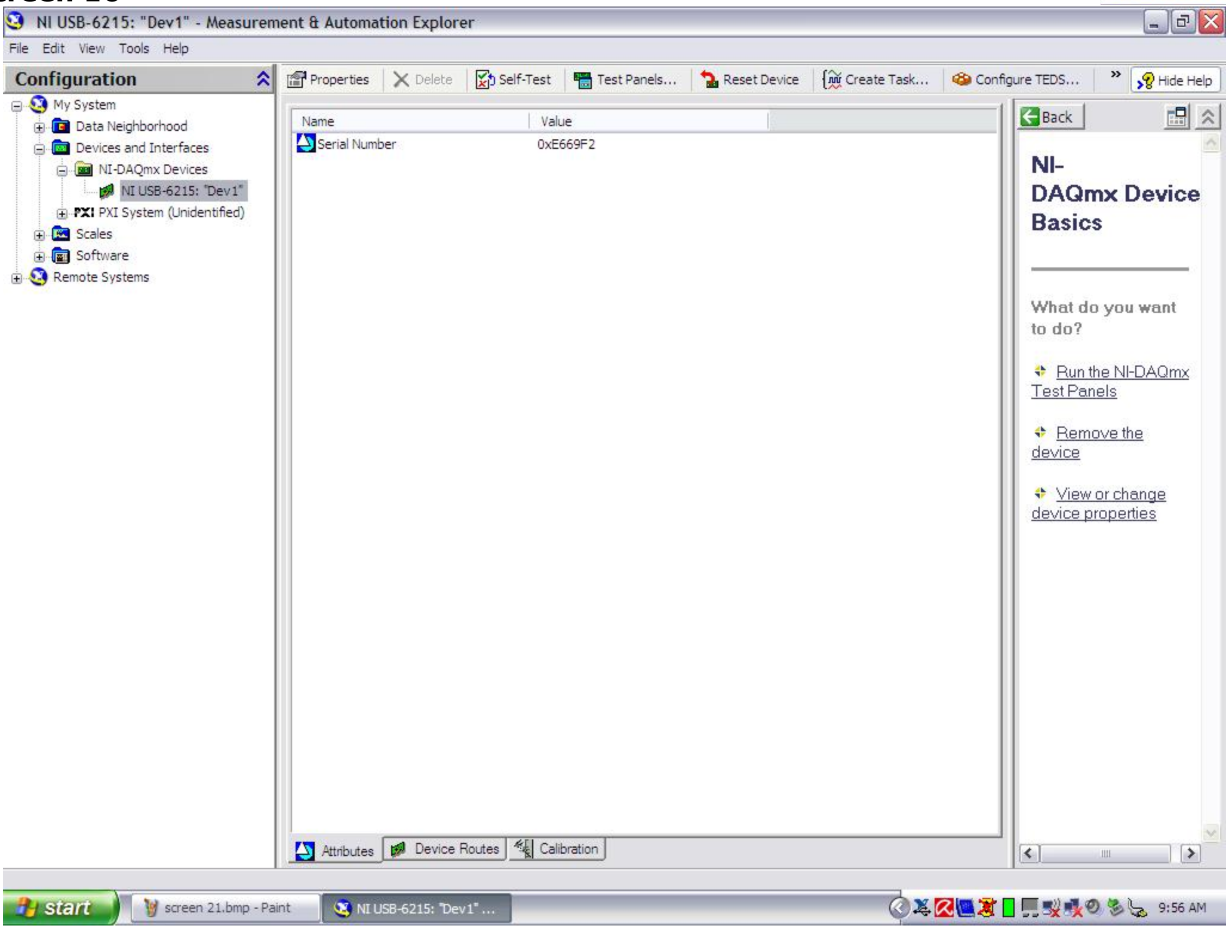
18. Click Finish.

Screen 15



19. Open Measurement & Automation by following the folder hierarchy shown on Screen 15.

Screen 16



Make sure the NI USB-6215 is installed as device name ”Dev1”. If so the DAQ-1 instrument is now properly correct installed and ready for use with AutoResp™ 1.

20. Remove CD labelled AutoResp™ 1.

3.4 Fiber optic oxygen instrument

3.4.1. Front side

OXYGEN SENSOR: Fiber optic oxygen input for chamber.

Connect an oxygen sensor, e.g. oxygen dipping probe, to this input.

TEMP: Fiber optic temperature input for ambient water.

Connect a temperature sensor, e.g. Pt100 probe, to this input.

Power button: Turns instrument on and off.

Power LED: Indicates instruments ON/OFF.

- Green means ON
- Yellow means sleep mode
- Red means OFF, but power connector is still ON
- Dark means Power connector is OFF

3.4.2. Back side

12 VDC: Power input for the 3 instrument.

Connect the instrument to the power via the AC/DC power adapter.

RS232: RS-232 interface (male).

Connect the instrument to the PC via the accompaying RS-232 cable.

For further information about the fiber optic oxygen instrument please look in the accompanying instruction manual or see 0
Fiber optic oxygen instrument instruction manual, page 53.

4. Introduction to intermittent flow respirometry

Three different methods are commonly used for measuring oxygen consumption in water breathers, e.g. fish:

- Closed respirometry
- Flow-through respirometry
- Intermittent flow respirometry

4.1 Closed respirometry (or constant volume respirometry)

Measurements are in a sealed chamber of known volume (a closed *respirometer*). The oxygen content of the water is measured initially (t_0), then the respirometer is closed and at the end of the experiment (t_1) the oxygen content is measured again.
Knowing the body weight of the animal, the respirometer volume and the oxygen content of the water at time t_0 and t_1 the mass specific oxygen consumption rate can be calculated as follows:

$$VO_2 = ([O_2]t_0 - [O_2]t_1) \cdot V/t \cdot BW^{-1}$$

VO_2	= oxygen consumption rate (mg O_2/kg/hour)
$[O_2]t_0$	= oxygen concentration at time t_0 (mg O_2/liter)
$[O_2]t_1$	= oxygen concentration at time t_1 (mg O_2/liter)
V	= respirometer volume minus volume of experimental animal (liter)
t	= $t_1 - t_0$ (hour)
BW	= body weight of experimental animal (kg)

An advantage of this method is its simplicity. A disadvantage is that the measurements are never made at a constant oxygen level, due to the continuous use of oxygen by the animal inside the respirometer. This might cause problems when interpreting data, since animal respiration often changes with ambient oxygen partial pressure.
Furthermore, metabolites from the experimental animal, *i.e.* CO_2, accumulate in the water, thus limiting the duration of measurements. This limited time for measurements prevents the experimental animal to recover from initial handling stress that often increase fish respiration significantly and for several hours, thus overestimating oxygen consumption rates.

4.2 Flow-through respirometry (or open respirometry)

This is a more sophisticated method for oxygen consumption measurements. Experimental animals are placed in a flow-through chamber, with known flow rate. Oxygen is measured in the inflow and outflow and oxygen consumption rate can be calculated as:

$VO_2 = F \cdot ([O_2]_{in} - [O_2]_{out}) / BW$

VO_2 = oxygen consumption rate (mg O_2/kg/hour)
F = water flow rate (l/hour)
$[O_2]_{in}$ = oxygen content in water inflow (mg O_2/liter)
$[O_2]_{out}$ = oxygen content in water outflow (mg O_2/liter)
BW = body weight of experimental animal (kg)

The advantages of this method are several:

1) the duration of the experiment is in principle unlimited
2) no accumulation of CO_2 and other metabolites
3) its possible to measure at a constant oxygen level
4) by controlling the quality of the inflowing water it's possible to measure metabolism at different desired levels of oxygen, salinity etc.

However, this method bring along one significant disadvantage: in order to determine oxygen consumption by *open respirometry* it is crucial that the system is in steady state. This means that the oxygen content of the in flowing and out flowing water, AND the oxygen consumption of the animal have to be constant.
If the oxygen consumption of the animal for some reason changes during the experiment, steady state will not exist for a while. Not until the system is in steady state again will the above formula give the correct oxygen consumption rate. The duration of the time lag depends on the relationship between chamber volume and flow rate. Thus, *open respirometry* measurements have poor time resolution and are not suitable for determination of oxygen consumption on organisms with a highly variable respiration like fish.

4.3 Intermittent flow respirometry (or open-closed respirometry)

Our systems for automatic respirometry works by *intermittent flow respirometry* aiming at combining the best of both 1) c*losed* and 2) *flow-through* respirometry.

Reference: Steffensen, J.F. (1989). Some errors in respirometry of aquatic breathers: how to avoid and correct for them. *J. Fish. Physiol. Biochem.* **6**; 49-59.

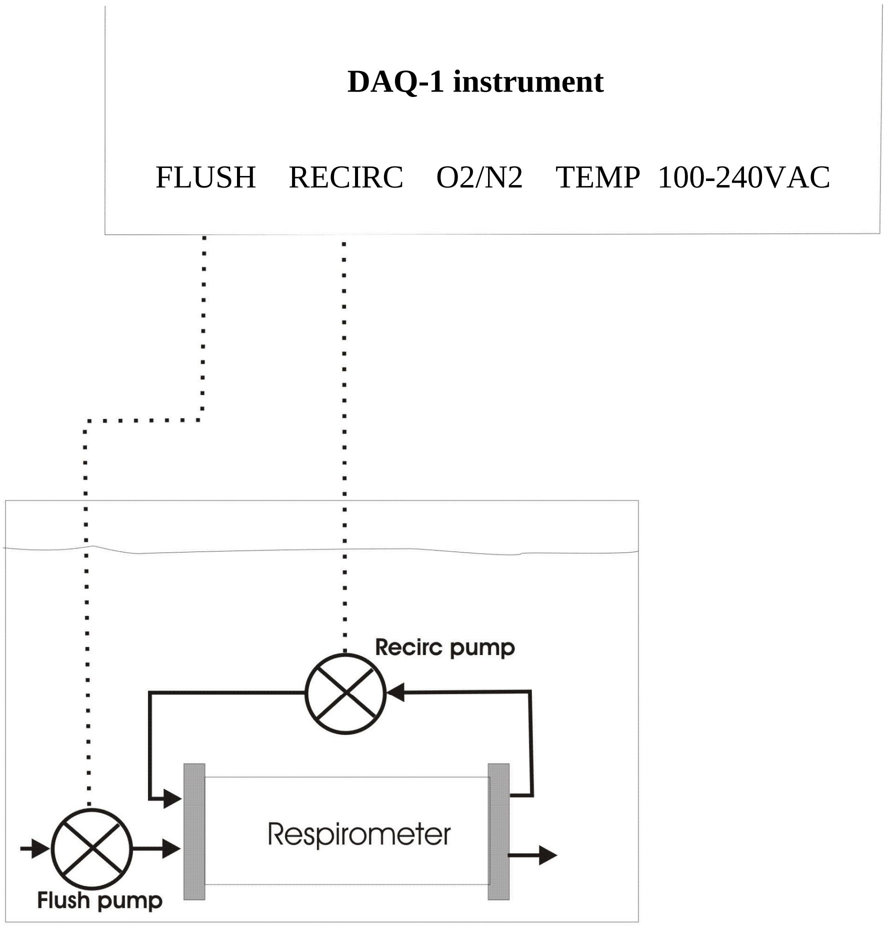
The experimental animal is placed in a closed chamber (respirometer) immersed in an ambient tank.
A recirculation pump ensures proper mixing of the water inside the respirometer and adequate flow past the oxygen probe. A second pump can change the water inside the respirometer with ambient water.
During measurements of oxygen consumption, this *flush pump* is turned off and the systems operates like 1) *closed respirometry*. Then the pc controlled flush pump turns on pumping ambient water into the respirometer and bringing the oxygen content back to pre measurement values.

In this way, problems with accumulating metabolites and severe changes in oxygen level due to animal respiration are avoided.

As with open respirometry, the duration of the experiment is in principle unlimited.

However, the most important advantage is the great time resolution of this method. Oxygen consumption rates of animals can be determined for every 10th minutes over periods of hours or days, making the system extremely suited for uncovering short term variations (minutes) in metabolism. In summary, our systems for respirometry are developed for prolonged and automatic measurements of oxygen consumption rate in a controlled laboratory environment.

Screen 17



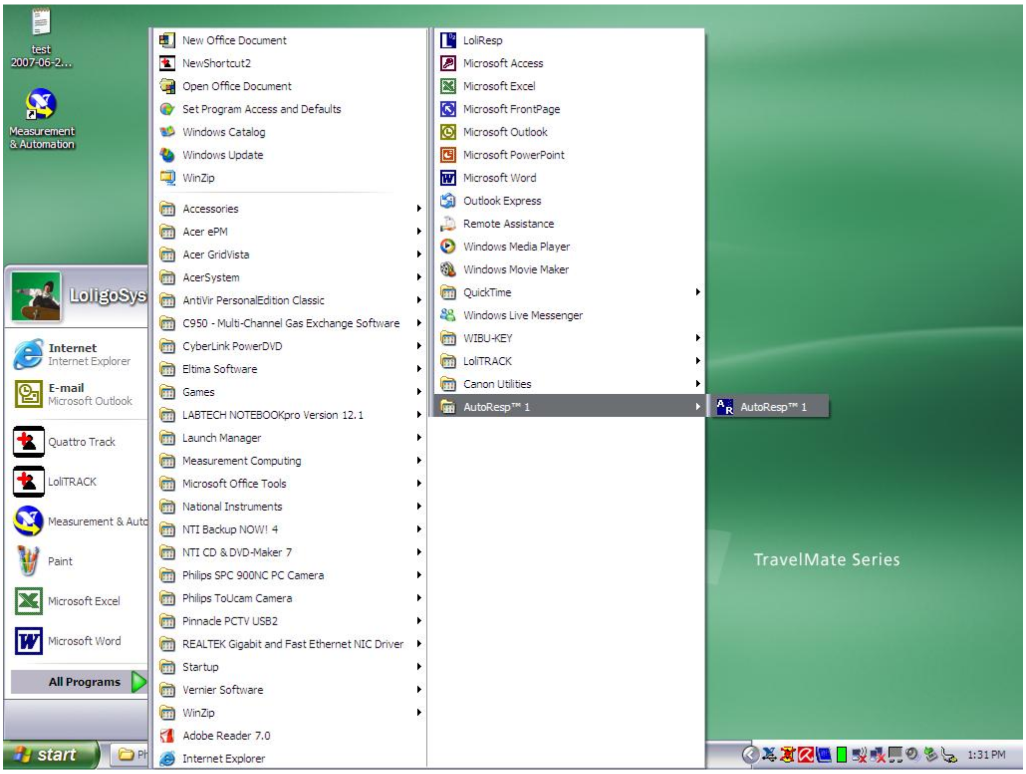
Screen 17 shows how to connect the flush and the recirc pump to the respirometer by tubes. The connection to the DAQ-1 instrument (dotted lines) is described above.

5. Using AutoResp™ 1

5.1 Startup

- Click on the AutoResp™ 1 icon in the Start menu by following the folder hierarchy shown on Screen 18

Screen 18

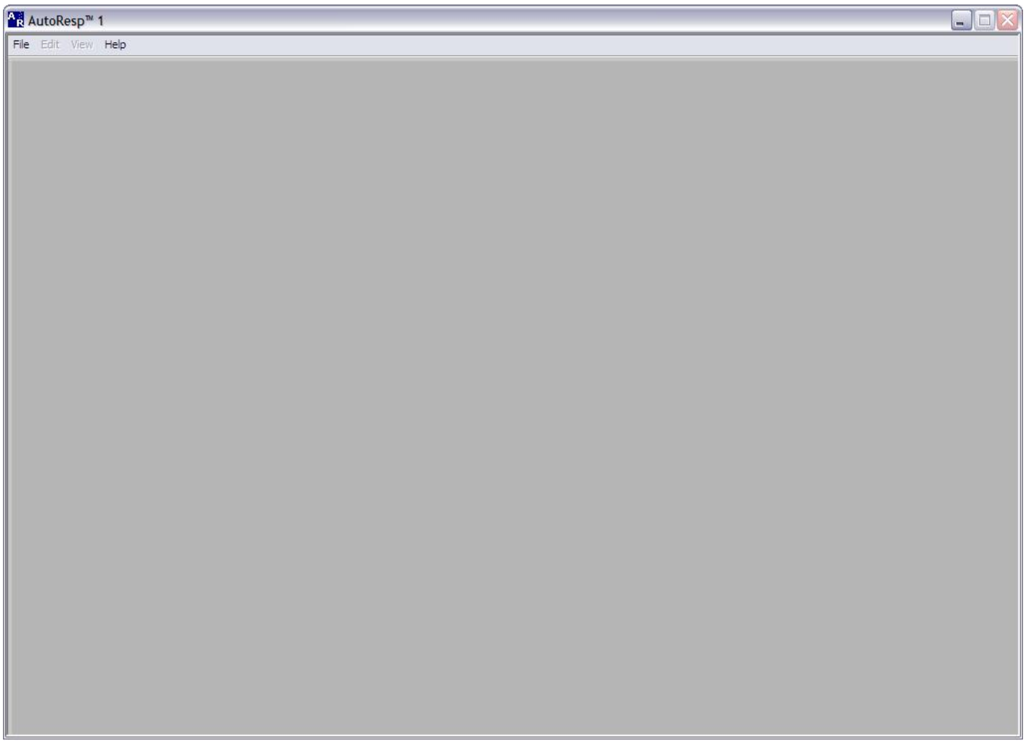


- If the USB hardware protection dongle is not connected to the PC, an error dialog box pops up, see Screen 19.

Screen 19

- Connect the USB hardware protection dongle to the PC via an USB port and click on Retry. Screen 20 now becomes present.

Screen 20



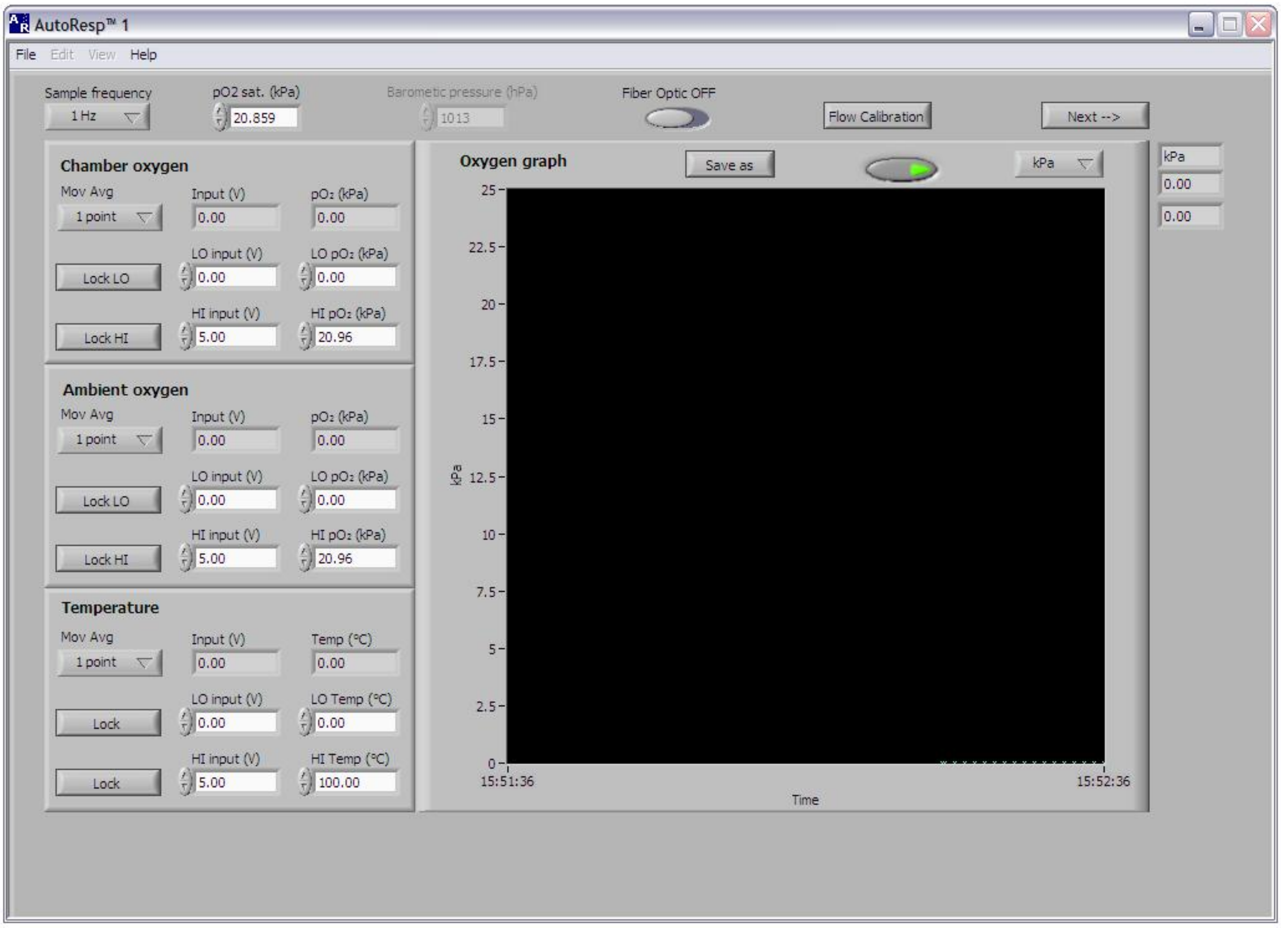
- When AutoResp™ 1 has started properly click on the File menu → New experiment. Screen 21 is now present.

5.2 *Calibration and selection of oxygen input*

5.2.1. General about Screen 21

Screen 21 is the calibration screen as it looks if chamber oxygen input is a 0-5 VDC analog signal, e.g. from an OXY-REG or some other O_2 instrument. On this screen a common sample frequency for all inputs can be chosen, and the inputs can be calibrated from voltages into oxygen partial pressures and temperature. This screen can also be used to do a quick oxygen or temperature measurement. **Please note:** The calibration values is not stored until an experiment has been started. That means the calibration values are NOT stored when the Next --> button is pressed.

Screen 21



5.2.2. Sample frequency button

Use this button to set a common sample frequency for all inputs. The frequency can be set from one sample to ten samples per second.
Please note: All inputs are always sampled at the same rate.

5.2.3. Barometic pressure (hPa)

Use this button to set the days actual barometic pressure in hPa. This field is used only in fiber optic measurements, and is disabled/enabled together with the fiber optic button status OFF/ON. The default value is 1013 = 1 atm = 760 mmHg.

5.2.4. pO_2 sat. (kPa)

Use this field to set the saturated partial pressure of oxygen in kPa, see 8.1 pO2 kPa table, page 48. This field is needed to convert the oxygen values in kPa to %air sat. and %oxygen sat. via the Unit button, see 5.2.11.2 Unit button, page 30. The default value is 20,859 which corresponds to 1013 kPa at 15 °C.

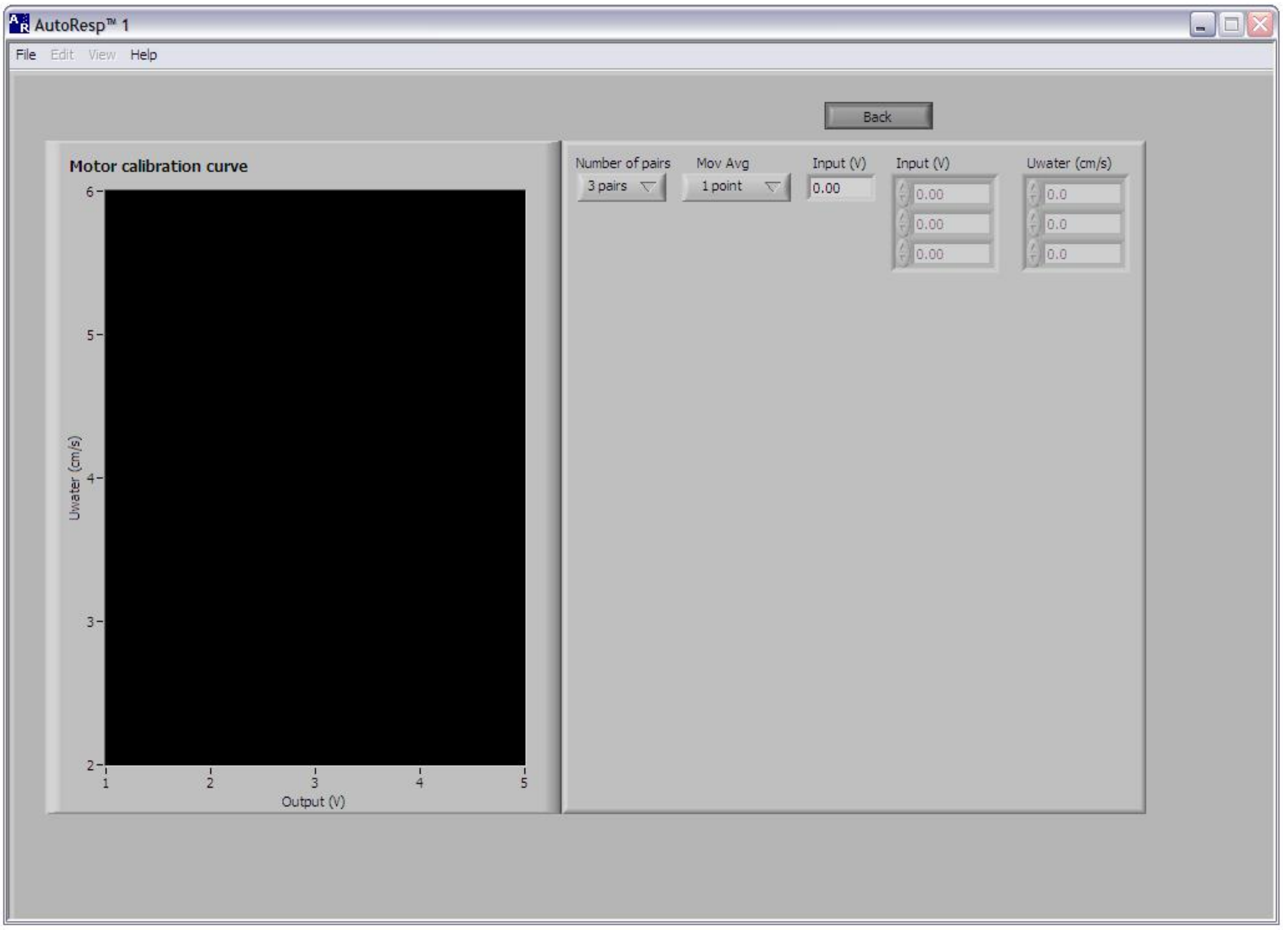
5.2.5. Fiber optic button

Use this button to select the type of oxygen input. When the button displays "Fiber optic off", chamber oxygen input should be 0-5 VDC, e.g. from OXY-REG instrument. When the button displays "Fiber optic on", the chamber oxygen input is acquired via the PreSens fiber optic instrument and the RS232 port of the PC. Pressing the button toggles Screen 21 and Screen 24. Before proceeding to "Fiber Optic on" the user is prompted to choose the COM port the fiber optic instruments from PreSens is connected too.
Please note: When fiber optic is on, as seen on Screen 24, the sample frequency cannot be altered as it is predetermined by the fiber optic instrument from PreSens. The frequency is approx. 1 Hz.

5.2.6. Flow Calibration

Use this button to enter the flow calibration menu as seen on Screen 22, page 24. To leave the calibration menu press on Back.

Screen 22



5.2.6.1. Number of pairs button

Click this button to select the number of calibration pairs [Input (V); Uwater(cm/s)] that should be used to calibrate the system.

5.2.6.2. Mov Avg button

Use this button to select if the motor input signal should be averaged or not. The default value is 1 point which means no averaging of the input. If any other value is chosen the chamber oxygen input will be averaged and the averaged values will be used for all subsequent computations, screen and file outputs. Use moving average if you wish to "smoothen" the motor input signal.

5.2.6.3. Input (V) field

Use this field to read the actual motor input in Volt.

5.2.6.4. Input (V) cells

In these cells the output of the motor controller instrument can be set as an input. If one "Input (V)" cell is set, the corresponding "Uwater (cm/s)" cell must be set in order to calibrate the system.
Please note: All visible cells must be filled out to make a proper calibration.

5.2.6.5. Uwater (cm/s) fields

In these cells the velocity of the water, e.g measured with a Höntzsch handheld HFA flow meter, can be set. If one "Uwater (cm/s)" cell is set, the corresponding "Input (V)" cell must be set in order to calibrate the system.
Please note: All visible cells must be filled out to make a proper calibration.

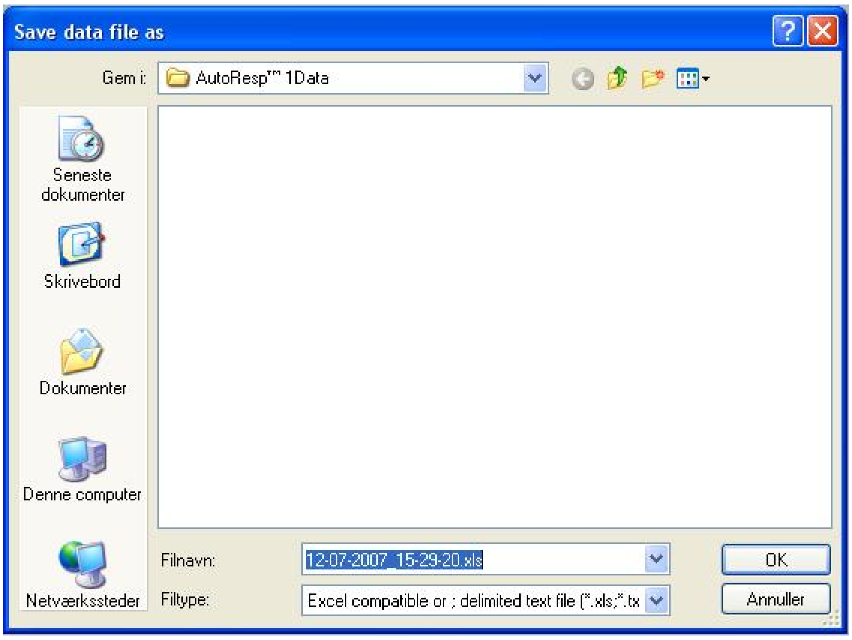
5.2.6.6. Motor calibration curve

The motor calibration curve is dynamically updated as the values in the two fields "Input (V)" and "Uwater (cm/s)" are changed. The calibration points are visible as white squares, and the best linear fit of the calibration points is visible as a straight line. The equation for the best linear fit is used to translate all subsequent output voltages of the motor into Uwater (cm/s).

5.2.7. Next --> button

Use this button to accept the calibration settings and move on to setting up a resp. experiment see 5.3 Setup experiment, page 35. Before proceeding to the settings setup the user is prompted to enter a data file name and path, see Screen 23 The path specifies the location of the main data file and a folder containing raw data files from the experiment. The path is by default the installation directory of AutoResp1\AutoResp1Data. The current time and date.xls is suggested as a filename in the prompt. Both the path and filename can be altered by choice. The system suggests two file extensions .xls and .txt. The .xls extension will create a tabulator separated text file which is readable by Microsoft ® Excel. The .txt extension will create a semicolon separated text file which is readable by any Microsoft ® Windows PC.
In addition to the data file the system will also generate a folder named as the data file. The dot (.) in the filename will however be replaced by an underscore (_) in the folder name. In this folder files containing raw data will be generated for each measurement loop. The raw data files contain data from the measure period on which the calculated values in the main data file are based.

Screen 23



5.2.8. Chamber oxygen field

5.2.8.1. Mov Avg button

Use this button to select if the chamber oxygen input signal should be averaged or not. The default value is 1 point which means no averaging of the input. If any other value is chosen the chamber oxygen input will be averaged and the averaged values will be used for all subsequent computations, screen and file outputs. Use moving average if you wish to "smoothen" the chamber oxygen signal.
Please note: No calibrated values will be displayed either on screen or in the data file before sufficient samples have been collected to compute the moving average. *E.g. if the moving average is set to 30 points and the sample rate is set to 10 Hz there will be no chamber oxygen values the first 30 samples equivalent to the first three seconds of samples.*

5.2.8.2. Input (V) indicator

This field displays the actual voltage input.

5.2.8.3. pO_2 (kPa) indicator

This field displays the actual calculated oxygen partial pressure from the voltage input.

5.2.8.4. Lock LO button

This button can be used to copy the actual value from the Input (V) indicator to the LO input (V) field instead of manually reading and retyping the value.

5.2.8.5. LO input (V)

Use this field to enter the low input voltage from the Input (V) indicator during the chamber oxygen calibration.
Please note: The field is limited to values between 0V to 5V

5.2.8.6. LO pO_2 (kPa)

Use this field to enter the oxygen pressure during the low level oxygen calibration of the chamber oxygen input.
Please note: The field is limited to values between 0.00 kPa to 60.00 kPa

5.2.8.7. Lock HI button

This button can be used to copy the actual value from the Input (V) indicator to the HI input (V) field instead of manually reading and retyping the value.

5.2.8.8. HI input (V)

Use this field to enter the high input voltage from the Input (V) indicator during the chamber oxygen calibration.
Please note: The field is limited to values between 0V to 5V

5.2.8.9. HI pO_2 (kPa)

Use this field to enter the oxygen pressure during the high level oxygen calibration of the chamber oxygen input.
Please note: The field is limited to values between 0.00 kPa to 60.00 kPa

5.2.9. Ambient oxygen field

5.2.9.1. Mov Avg button

Use this button to select if the ambient oxygen input signal should be averaged or not. The default value is 1 point which means no averaging of the input. If any other value is chosen the ambient oxygen input will be averaged and the averaged values will be used for all subsequent computations, screen and file outputs. Use moving average if you wish to "smoothen" the ambient oxygen signal.
Please note: No calibrated values will be displayed either on screen or in the data file before sufficient samples have been collected to compute the moving average. *E.g. if the moving average is set to 30 points and the sample rate is set to 10 Hz there will be no ambient oxygen values the first 30 samples equivalent to the first three seconds of samples.*

5.2.9.2. Input (V) indicator

This field displays the actual voltage input.

5.2.9.3. pO_2 (kPa) indicator

This field displays the actual calculated oxygen partial pressure from the voltage input.

5.2.9.4. Lock LO button

This button can be used to copy the actual value from the Input (V) indicator to the LO input (V) field instead of manually reading and retyping the value.

5.2.9.5. LO input (V)

Use this field to enter the low input voltage from the Input (V) indicator during the ambient oxygen calibration.
Please note: The field is limited to values between 0V to 5V

5.2.9.6. LO pO_2 (kPa)

Use this field to enter the oxygen pressure during the low level oxygen calibration of the ambient oxygen input.
Please note: The field is limited to values between 0.00 kPa to 60.00 kPa

5.2.9.7. Lock HI button

This button can be used to copy the actual value from the Input (V) indicator to the HI input (V) field instead of manually reading and retyping the value.

5.2.9.8. HI input (V)

Use this field to enter the high input voltage from the Input (V) indicator during the ambient oxygen calibration. The lower Lock button can be used to copy the actual value from the Input (V) indicator to the HI input (V) field instead of manually reading and retyping the value.
Please note: The field is limited to values between 0V to 5V

5.2.9.9. HI pO_2 (kPa)

Use this field to enter the oxygen pressure during the high level oxygen calibration of the ambient oxygen input.
Please note: The field is limited to values between 0.00 kPa to 60.00 kPa

5.2.10. Temperature field

5.2.10.1. Mov Avg button

Use this button to select if the temperature input signal should be averaged or not. The default value is 1 point which means no averaging of the input. If any other value is chosen the temperature input will be averaged and the averaged values will be used for all subsequent computations, screen and file outputs. Use moving average if you wish to "smoothen" the temperature signal.
Please note: No calibrated values will be displayed either on screen or in the data file before sufficient samples have been collected to compute the moving average. *E.g. if the moving average is set to 30 points and the sample rate is set to 10 Hz there will be* temperature *values the first 30 samples equivalent to the first three seconds of samples.*

5.2.10.2. Input (V) indicator

This field displays the actual voltage input.

5.2.10.3. Temp (°C) indicator

This field displays the actual calculated temperature from the voltage input.

5.2.10.4. Lock LO button

This button can be used to copy the actual value from the Input (V) indicator to the LO input (V) field instead of manually reading and retyping the value.

5.2.10.5. LO input (V)

Use this field to enter the low input voltage from the Input (V) indicator during the low temperature calibration.
Please note: The field is limited to values between 0V to 5V.

5.2.10.6. LO Temp (°C)

Use this field to enter the temperature during the low temperature calibration.
Please note: The field is limited to values between 0 °C to 100 °C.

5.2.10.7. Lock HI button

This button can be used to copy the actual value from the Input (V) indicator to the HI input (V) field instead of manually reading and retyping the value.

5.2.10.8. HI input (V)

Use this field to enter the high input voltage from the Input (V) indicator during the high temperature calibration.
Please note: The field is limited to values between 0V to 5V.

5.2.10.9. HI Temp (°C)

Use this field to enter the temperature during the high temperature calibration.
Please note: The field is limited to values between 0 °C to 100 °C.

5.2.11. Oxygen graph field

This graph shows the oxygen pressure vs. time measured going back 1 min. By right clicking the graph it is possible to show/hide the ambient oxygen and export a simplified image of the graph.
Right to the graph there are three fields, where current data are shown. The first field shows the chosen units, which can be altered by the unit button.

5.2.11.1. Save button

Use this button to save the data displayed in the Oxygen graph. Furthermore the temperature will be saved. When pressed the user is prompted to enter a file name and path, see Screen 23. The path is by default the installation directory of AutoResp1\AutoResp1Data. The current time and date.xls is suggested as a filename in the prompt. Both the path and filename can be altered by choice. The system suggests two file extensions .xls and .txt. The .xls extension will create a tabulator separated text file which is readable by Microsoft ® Excel. The .txt extension will create a semicolon separated text file which is readable by any Microsoft ® Windows PC.

5.2.11.2. Unit button

Use this button to convert the displayed oxygen partial pressure values on the chamber oxygen graph into Torr, %oxygen saturation or %air saturation.

5.2.11.3. Play button

Use this button to start/stop data from being graphed. When the play button is ON, new data will be shown on the oxygen graph and the Unit button is enabled. When the play button is OFF, the Unit button is disabled. Furthermore a scroll bar is shown for the chamber oxygen graph.

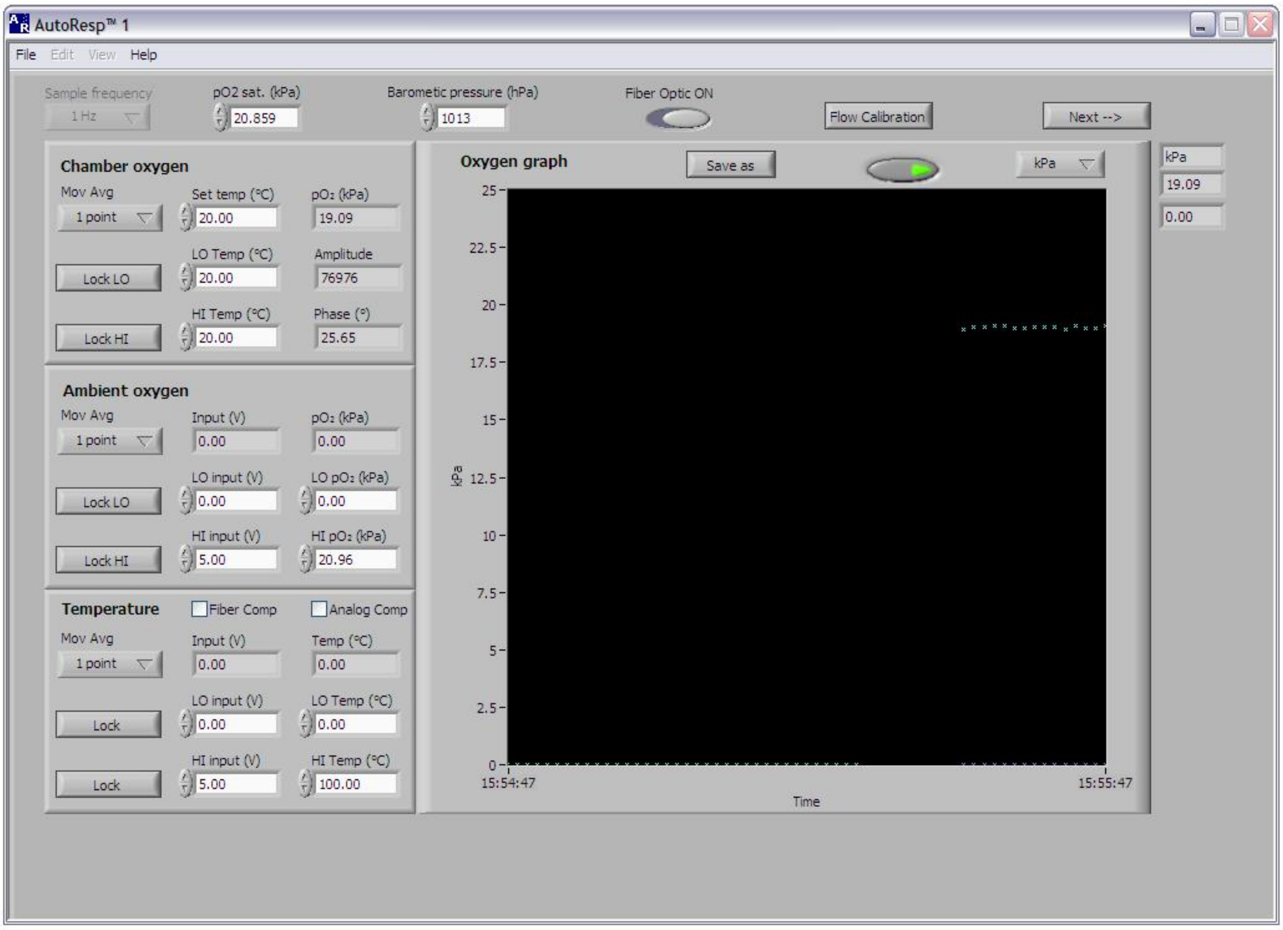
5.2.12. General about Screen 24

Screen 24 is the calibration screen as it looks if the chamber oxygen signal is acquired from the fiber optic oxygen instrument from PreSens and the RS232 port of the PC.

Please note: When fiber optic is on, as seen on Screen 24, the sample frequency cannot be altered as it is predetermined to approx. 1 Hz by the fiber optic oxygen instrument.

Please note: The look and functionality of the Ambient oxygen field and the Chamber oxygen graph field are similar to those of Screen 21.

Screen 24



5.2.13. Chamber oxygen field

5.2.13.1. Mov Avg button

Use this button to select if the chamber oxygen input signal should be averaged or not. The default value is 1 point which means no averaging of the input. If any other value is chosen the chamber oxygen input will be averaged and the averaged values will be used for all subsequent computations, screen and file outputs. Use moving average if you wish to "smoothen" the chamber oxygen signal.
Please note: No calibrated values will be displayed either on screen or in the data file before sufficient samples have been collected to compute the moving average. *E.g. if the moving average is set to 10 points there will be no chamber oxygen values the first ten samples equivalent to the first ten seconds of samples.*

5.2.13.2. Set temp. (°C)

Use this field to specify the temperature of the water in the chamber. If the temperature cannot be kept during measurements within the entered value with ±0,2 °C, choose to compensate the fiber optic oxygen signal with the measured temperature signal. Do this either with the fiber optic measured temperature by enable the checkmark Fiber Comp, see 5.2.14.1 Fiber Comp, page 34 or the analog measured temperature by enable the checkmark Analog Comp, see 5.2.14.2 Analog Comp, page 34. If Analog Comp or Fiber Comp is enabled, the Set temp (°C) field gets disabled and will be updated with the measured temperature. The fiber optic oxygen value will now be real-time compensated for the temperature.

5.2.13.3. pO_2 (kPa) indicator

This field displays the chamber oxygen pressure from the fiber optic instrument from PreSens relative the calibration.

5.2.13.4. Lock LO button

Press this button to calibrate the fiber optic oxygen instrument in a 0% air saturation O_2 solution.
Please note: It is important not to push the Lock LO button before the Phase (°) and Amplitude indicators has stabilized.
Please note: A full calibration must consist of a 0% calibration and a 100% calibration before the oxygen readouts are reliable.
Please note: The calibration will be written down on an EEPROM in the fiber optic instrument. If you want to use the fiber optic instrument with the software from PreSens a new calibration may be done using this software.

5.2.13.5. LO Temp (°C)

Use this field to specify the temperature during calibration of the fiber optic instrument with the 0% air saturation O_2 solution.

5.2.13.6. Lock HI button

Press this button to calibrate the fiber optic instrument in a 100% air saturation O_2 solution.
Please note: It is important not to push the Lock HI button before the Phase (°) and Amplitude indicators has stabilized.
Please note: A full calibration must consist of a 0% calibration AND a 100% calibration before the oxygen readouts are reliable.
Please note: The calibration will be written down on an EEPROM in the fiber optic instrument. If you want to use the fiber optic instrument with the software from PreSens a new calibration may be done using this software.

5.2.13.7. HI Temp (°C)

Use this field to specify the temperature during calibration of the fiber optic instrument with the 100% air saturation O_2 solution.

5.2.13.8. Amplitude indicator

This field indicates the amplitude of the optical signal that the fiber optic oxygen instrument measures. The signal strength is an indicator for the sensor condition. See the manual of the fiber optic instrument from PreSens for further explanation.

5.2.13.9. Phase (°) indicator

This field indicates the phase angle of the optical signal that the fiber optic oxygen instrument uses for computations of the chamber oxygen pressure.

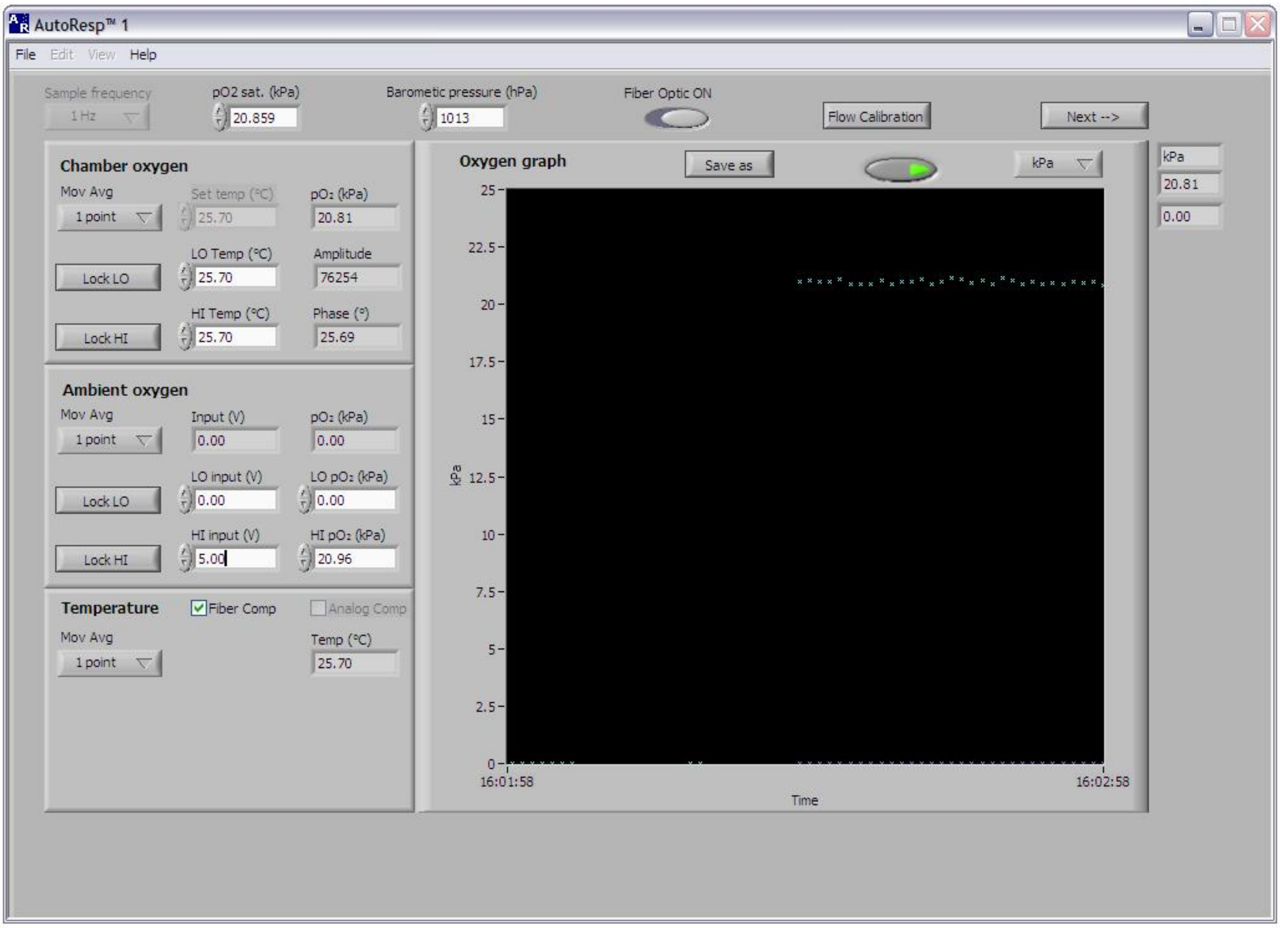
5.2.14. Temperature field

When the fiber optic button is ON, the Temperature field gets changed from Screen 21 page 22, to Screen 24 page 31. Two checkmarks appears named Fiber Comp and Analog Comp.

5.2.14.1. Fiber Comp

Use this checkmark to enable/disable the real-time compensation of the fiber optic oxygen signal with the measured temperature signal from the fiber optic instrument. If this checkmark gets enabled, the Temperature field changes as seen on Screen 25.

Screen 25



5.2.14.2. Analog Comp

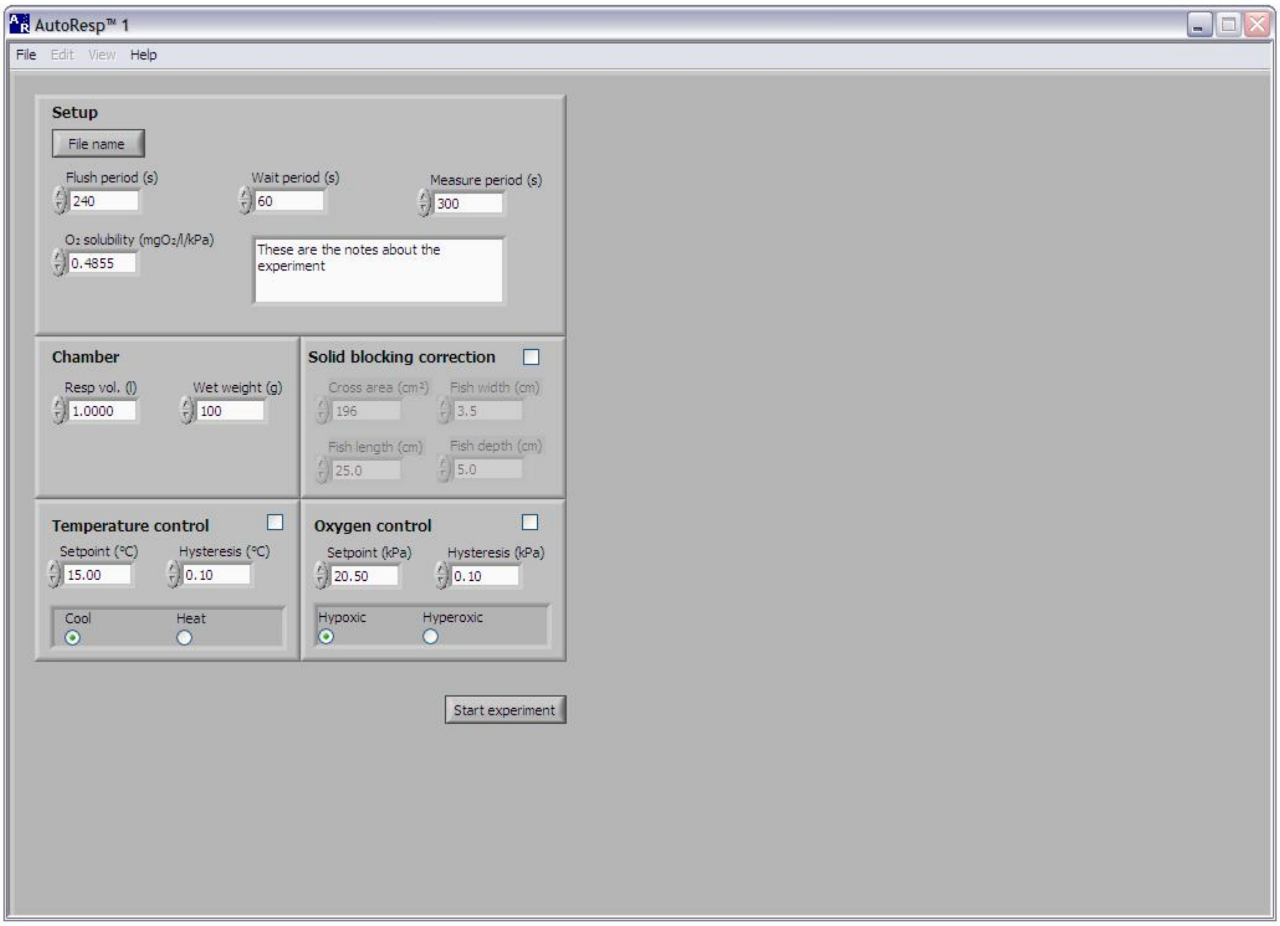
Use this checkmark to enable/disable the compensation of the fiber optic oxygen signal with the analog measured temperature. If the temperature changes with 0,2 °C, the new temperature will be send to the fiber optic instrument.

5.3 *Setup experiment*

5.3.1. General about Screen 26

On Screen 26 the intermittent respirometry experiment is configured.

Screen 26



5.3.1.1. File name button

Use this button to change the path and filename of the data file and raw data folder. The path specifies the location of the main data file and the folder containing raw data from the experiment. The path is by default the installation directory of AutoResp4\AutoResp4Data. The current time and date.xls is suggested as a filename in the prompt. Both the path and filename can be altered by choice. The system suggests two file extensions .xls and .txt. The .xls extension will create a tabulator separated text file which is readable by Microsoft ® Excel. The .txt extension will create a semicolon separated text file which is readable by any Microsoft ® Windows PC.
In addition to the data file the system will also generate a folder named as the data file. The dot (.) in the filename will however be replaced by an underscore (_) in the folder name. In this folder files containing raw data will be generated for each measurement loop. The raw data files contain data from the measure period on which the calculated values in the main data file are based.
Please note: The start experiment button will be disabled until a valid path/filename is selected.

5.3.2. Setup field

5.3.2.1. Flush period (s)

Use this field to specify the duration of flush periods in seconds during the intermittent flow respirometry experiment.
Please note: The field is limited to values between 0 s to 35 weeks.

5.3.2.2. Wait period (s)

Use this field to specify the duration of the wait periods in seconds during the intermittent flow respirometry experiment.
Please note: The field is limited to values between 1 s to 35 weeks.

5.3.2.3. Measure period (s)

Use this field to specify the duration of the measure periods during the intermittent flow respirometry experiment.
Please note: The field is limited to values between 1 s to 35 weeks.

5.3.2.4. O_2 solubility (mg O2/l/kPa)

Use this field to specify the oxygen solubility of sample water.
Please note: This field is limited to values between 0 to 0,75 mg/L/kPa. The default value is 0,4855 corresponding to a 15°C fresh water. For further information see the appendix, page 48 and 49.

5.3.2.5. Notes field

In this field the user can write a note about the experiment. The note is saved to the data file.

5.3.3. Chamber field

5.3.3.1. Resp vol. (l)

Use this field to specify the volume of water used for the calculation of the MO_2 value.

Resp vol. = chamber volume + tube volume - volume of organism(s)

To specify the Resp vol., fill the chamber and the tubes with water and measure the weight on a scale. Now empty the chamber and the tubes for water and measure the weight again. The difference between the two measurements will be the weight of the chamber volume and the tube volume. Multiply the calculated weight with the mass specific volume of water for finding the volume for the chamber and the tubes.
To find the volume of the organism measure the weight of the organism and multiply with the mass specific volume of the organism.

Please note: This field is limited to values between 0 to 2500 l.

5.3.3.2. Wet weight (g)

Use this field to specify the weight of the experimental organism(s).
Please note: This field is limited to values between 0,001g to 100 kg

5.3.4. Solid blocking correction field

An animal swimming in a channel obstructs the flow, causing water to run faster past the swimming animal. This results in a fractional error, i.e. a difference in water velocity depending on the presence and size of flow obstructing animals.

Choose Solid blocking correction to make the program correct for solid blocking effects according Bell & Terhune (1970):

Fractional error = 0.8 • 0.5 (BL/fish radius) • (fish square area/cross area)3/2

BL:	Body length of fish
Fish radius ("Thickness"):	(fish width + fish depth)/4
Fish square area:	PI(fish radius)2
Cross area:	cross area of swim tunnel working section

The solid blocking correction factor is then calculated as:

Solid blocking correction factor = 1/BL(fractional error +1)

and used to convert water velocity in cm/s (as measured during flow calibration with no fish in the working section) into relative swimming speed in BL/s during experiments with swimming fish.

Reference
Bell, W.H. & Terhune, L.D.B. (1970). Water tunnel design for fisheries research. Fish.Res.Bd.Can.Tech.Rep. 195, 1-69.

5.3.4.1. Checkbox

Use the checkbox to enable or disable the solid blocking correction.

5.3.4.2. Cross area (cm^2)

Use this field to specify the cross sectional area of the working section.

5.3.4.3. Fish length (cm)

Use this field to specify the length of the fish.

5.3.4.4. Fish width (cm)

Use this field to specify the width of the fish.

5.3.4.5. Fish depth (cm)

Use this field to specify the width of the fish.

5.3.5. Temperature control field

5.3.5.1. Checkbox

Use the checkbox to enable or disable the TEMP relay on the DAQ-1 instrument. If the checkbox is checked AutoResp™ 1 can control the temperature in the ambient water as specified in the temperature control field.

5.3.5.2. Setpoint (°C)

Use this field to set a temperature level in the ambient water that the system should maintain during an experiment.
Please note: The field is limited to values between -2 °C to 100 °C.

5.3.5.3. Hysteresis (°C)

Use this field to change the temperature hysteresis value. Use a hysteresis value > 0 °C to ensure that the cooler/heater pump does not flicker on the sharp edge of the setpoint.
If the hysteresis temperature = 0 °C the cooler/heater pump is very susceptible to electronic noise on the temperature channel.
Please note: The field is limited to values between 0 °C to 49,99 °C.

5.3.5.4. Cool/Heat

Use these buttons to select if the system must maintain the setpoint as a maximum (Cool) or a minimum (Heat) temperature.

5.3.6. Oxygen control field

5.3.6.1. Checkbox

Use the checkbox to enable or disable the O2/N2 relay on the DAQ-1 instrument. If the checkbox is checked AutoResp™ 1 can control the O_2 pressure in the ambient water as specified in the oxygen control field.

5.3.6.2. Setpoint (kPa)

Use this field to set an oxygen level in the ambient water that the system should maintain during an experiment.
Please note: The field is limited to values between 0 kPa to 60 kPa.

5.3.6.3. Hysteresis (kPa)

Use this field to change the oxygen hysteresis value. Use a hysteresis value > 0 kPa to ensure that the O_2/N_2 valve does not flicker on the sharp edge of the setpoint. If the hysteresis pressure is 0 kPa the O_2/N_2 valve is very susceptible to electronic noise on the ambient oxygen channel.
Please note: The field is limited to values between 0 kPa to 30 kPa.

5.3.6.4. Hypoxic/Hyperoxic

Use these buttons to select if the system must maintain the setpoint as a maximum (Hypoxic) or a minimum (Hyperoxic) oxygen pressure.

5.3.7. Start experiment button

Use this button to accept the setup settings as they are displayed on Screen 21, Screen 22, Screen 24, Screen 25 or Screen 26 and proceed to Screen 27.

5.3.8. General menus

5.3.8.1. File → Exit

Exits AutoResp™ 1 without saving changes to the calibration screens.

5.3.8.2. File → Continue experiment

Continues the last experiment, if stopped by user. Data on the graphs will only be in memory, when AutoResp™ 1 has not been shut down.
Please note: This option is only available if a calibration has been made and the user has stopped a running experiment.

5.3.8.3. Help → About

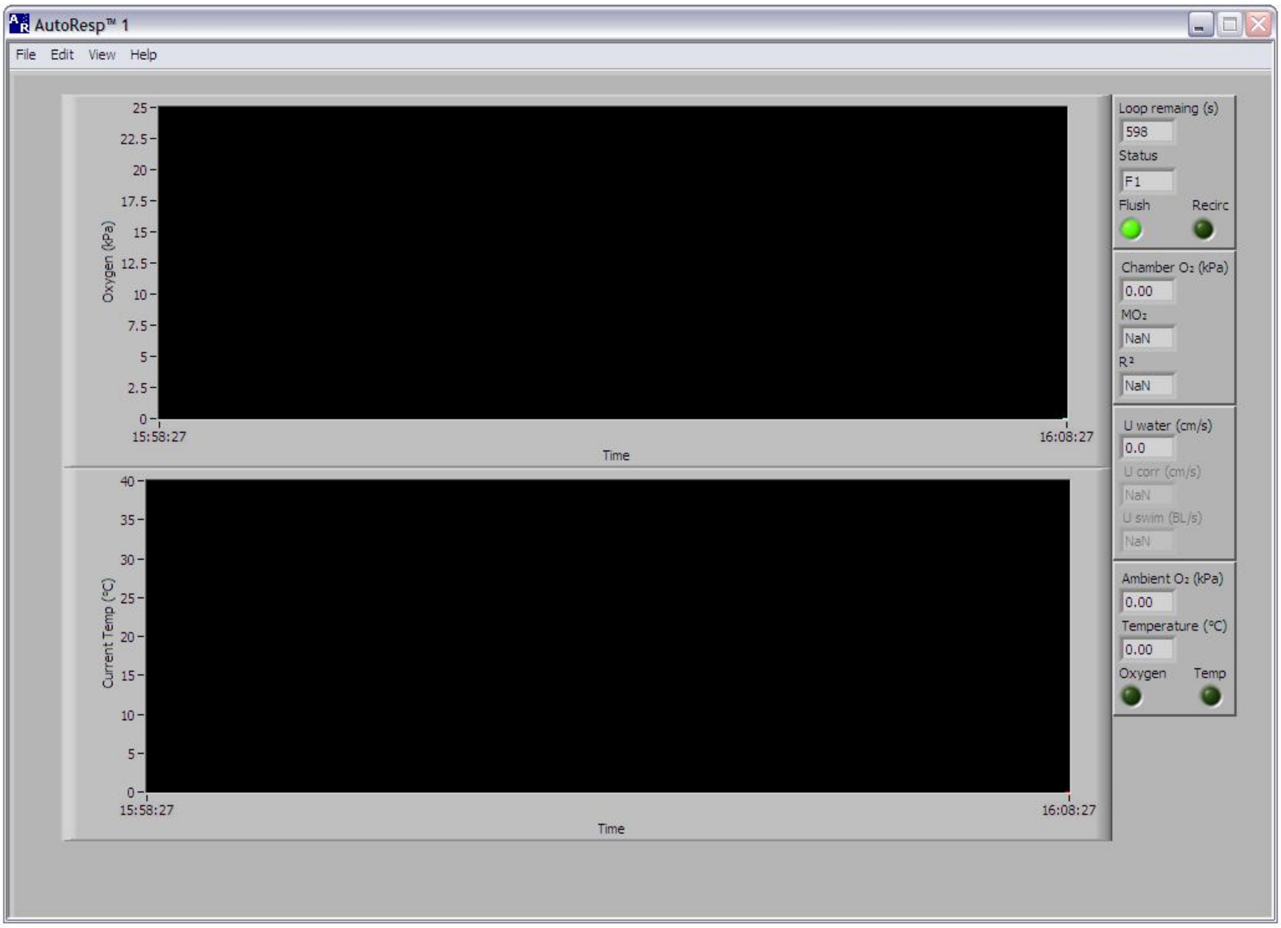
Displays contact information about Loligo Systems.

5.4 *Running experiment*

5.4.1. General about Screen 27

On Screen 27 the intermittent respirometry experiment is running.

Screen 27



5.4.2. Oxygen graph field

This field shows the measured oxygen values vs. time. By right clicking the graph, the user can choose to show/hide the ambient oxygen and modify the time interval on the x scale (10, 30 and 90 min). Another option is to save the graph to a file and to show the experiment settings.

5.4.3. Graph field

This field shows some different graphs for the experiment. As default the current temperature vs. time graph is shown. The user can choose between the following graphs by clicking with the mouse on the view button in the menu.

- MO_2 vs. time
- MO_2 vs. avg. Chamber O_2
- MO_2 vs. avg. Uwater
- MO_2 vs. Uswim
- Avg. temperature vs. time
- Current temperature vs. time
- R^2 vs. time
- Current R^2 vs. time

The MO_2, avg. temperature and the R^2 vs. time graph shows data from the whole experiment. The current temperature and the current R^2 vs. time graph shows data for the last 10, 30 or 90 min, depended on the x scale from the oxygen graph, see 5.4.2. By right clicking on the graph, the user can save the shown graph to a file.

5.4.4. Status field

5.4.4.1. Loop remaining (s)

This field shows the remaining seconds left of the current measurement loop. The loop is defined as the summed duration of flush, wait and measure periods.

5.4.4.2. Loop number (s)

This field shows the actual state of the loop and the number of loops. The letter F means flush, W means wait and M means measure. Behind the letter a number is shown, which shows the actual number of the loop.

5.4.4.3. Flush indicator

This diode indicates if the flush pump is activated or not. The flush pump is running only in a flush period.

5.4.4.4. Recirc indicator

This diode indicates if the recirculation pump is activated or not. The recirculation pump is running in a flush and a measure period.

5.4.5. Chamber measure field

5.4.5.1. Chamber O_2 (kPa)

This field shows the actual measured oxygen pressure for the chamber.

5.4.5.2. MO_2 field

This field shows the calculated MO_2 value for the chamber as described in background, see 4 Introduction to intermittent flow respirometry, page 16. The MO_2 value is calculated as follows:

$$MO_2\left[\frac{mg\,O_2}{h \cdot kg}\right] = 3600 \cdot O_2\,\text{solubility}\left[\frac{mgO_2}{l \cdot kPa}\right] \cdot slope\left[\frac{kPa}{s}\right] \cdot resp\,vol.[l] \cdot \frac{1000}{wet\,weight}\left[g^{-1}\right]$$

Where slope is calculated as follows:

$$slope\left[\frac{kPa}{s}\right] = \frac{O_{2\,n} - O_{2\,n-1}}{time_n - time_{n-1}}$$

Please note: MO_2 is only calculated in each measurement period, e.g. 29 MO_2 values after 30 seconds measure period at 1 Hz.

5.4.5.3. R^2 field

This field shows the calculated R^2 linear correlation coefficient value for the chamber when MO_2 is calculated. The R^2 value can be used to validate the linear fit of the O_2 curve.

5.4.6. Velocity field

5.4.6.1. U water (cm/s)

This field shows the actual measured velocity in centimeter per seconds.

5.4.6.2. U corr (cm/s)

This field shows the calculated corrected value. This value is only calculated if solid correction is enabled.

5.4.6.3. U swim (BL/s)

This field shows the actual measured velocity in body length per seconds. This value is only calculated if solid correction is enabled.

5.4.7. Ambient measure field

5.4.7.1. Ambient O_2 (kPa)

This field shows the actual measured ambient water oxygen pressure.

5.4.7.2. Oxygen regulation

This field shows if the oxygen regulator is working to maintain the oxygen level in the ambient tank as hypoxic or hyperoxic, see 5.3.6 Oxygen control field, page 38.

5.4.7.3. Temperature (°C)

This field shows the actual measured temperature.

5.4.7.4. Temperature regulation

This field shows if the temperature regulator is working to maintain the temperature level in the ambient water, see 5.3.5 Temperature control field, page 38.

5.4.8. Menus in Screen 27

5.4.8.1. File → Stop experiment

This option stops the current experiment.
Please note: To immediately turn on the flush pump use this option.

5.4.8.2. Edit → Oxygen

While the experiment is running the user can use this option to alter the oxygen regulation. This can be done by changing the setpoint, the hysteresis or the mode hypoxic/hyperoxic.

5.4.8.3. Edit → Temperature

While the experiment is running the user can use this option to alter the temperature regulation. This can be done by changing the setpoint, the hysteresis or the mode cool/heat.

5.4.8.4. View

By this option the user decides which graph is to be shown in the graph field, see 5.4.3 Graph field, page 41.

5.4.8.5. Help → About

Displays contact information about Loligo Systems

6. Troubleshooting

6.1 No connection to the DAQ-1 instrument

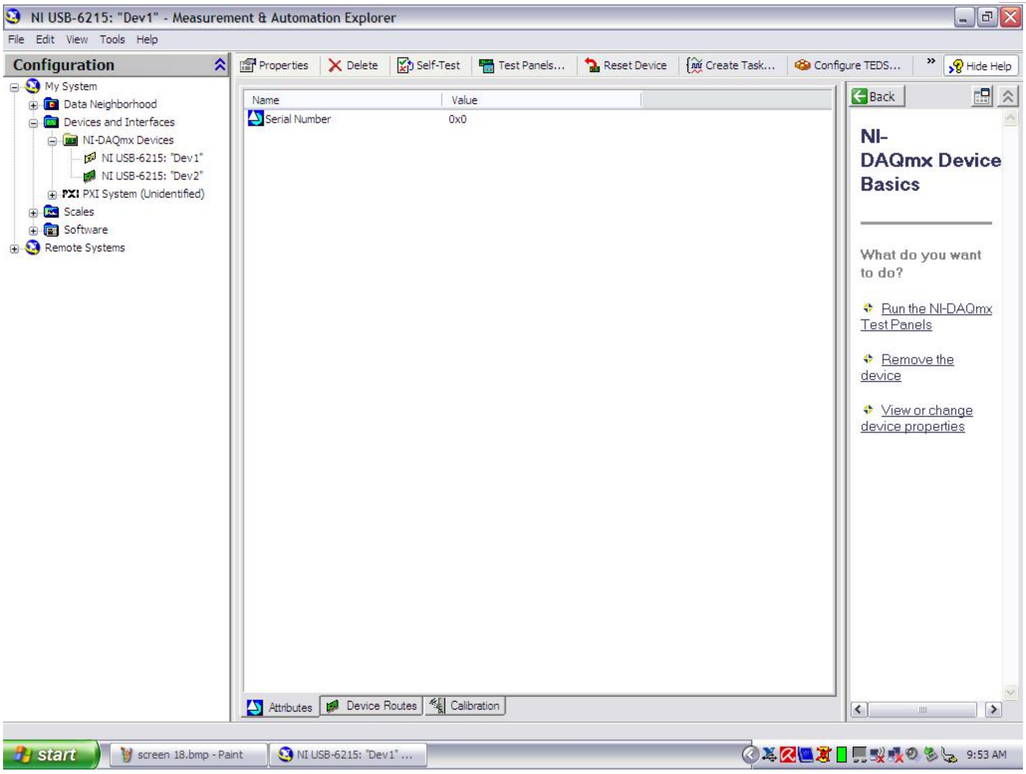
Screen 28

The error shown on Screen 28 occurs, when the NI-USB 6215 DAQ card has no properly connection to the PC. This error can occur in 2 ways.

6.1.1. Name is not "dev1"

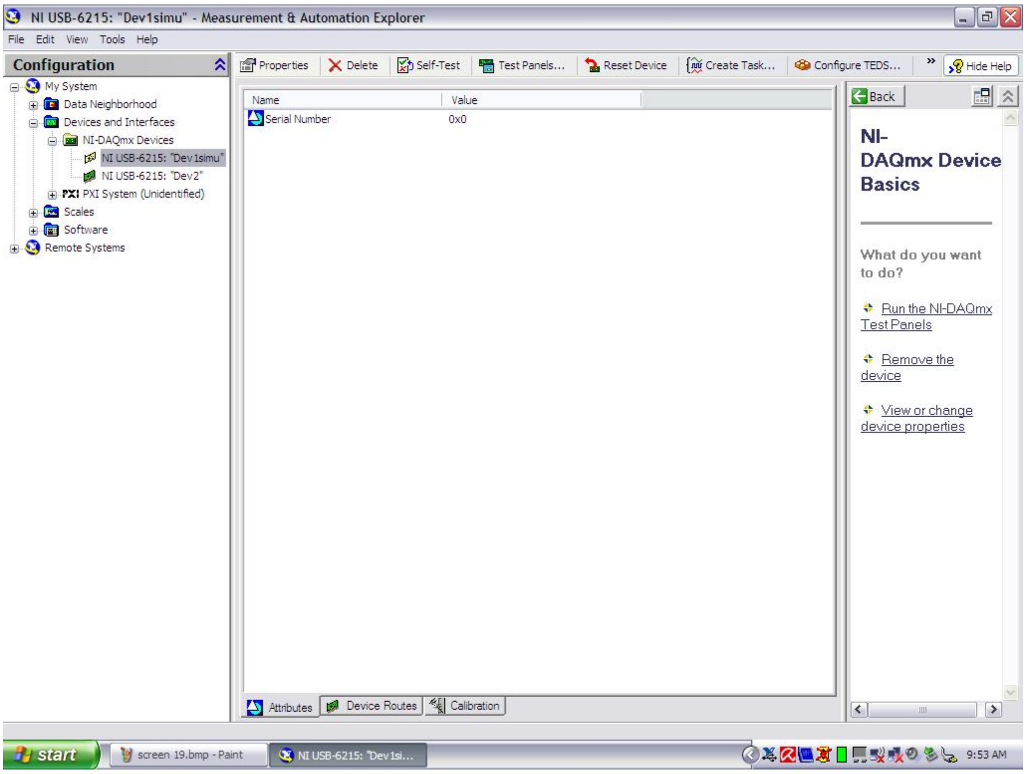
To change the device name, open Measurement & Automation by following the folder hierarchy shown on Screen 15, page 13. The following example shows on Screen 29 a simulated device named as "dev1". The NI-USB 6215 DAQ card is registered as "dev2"

Screen 29



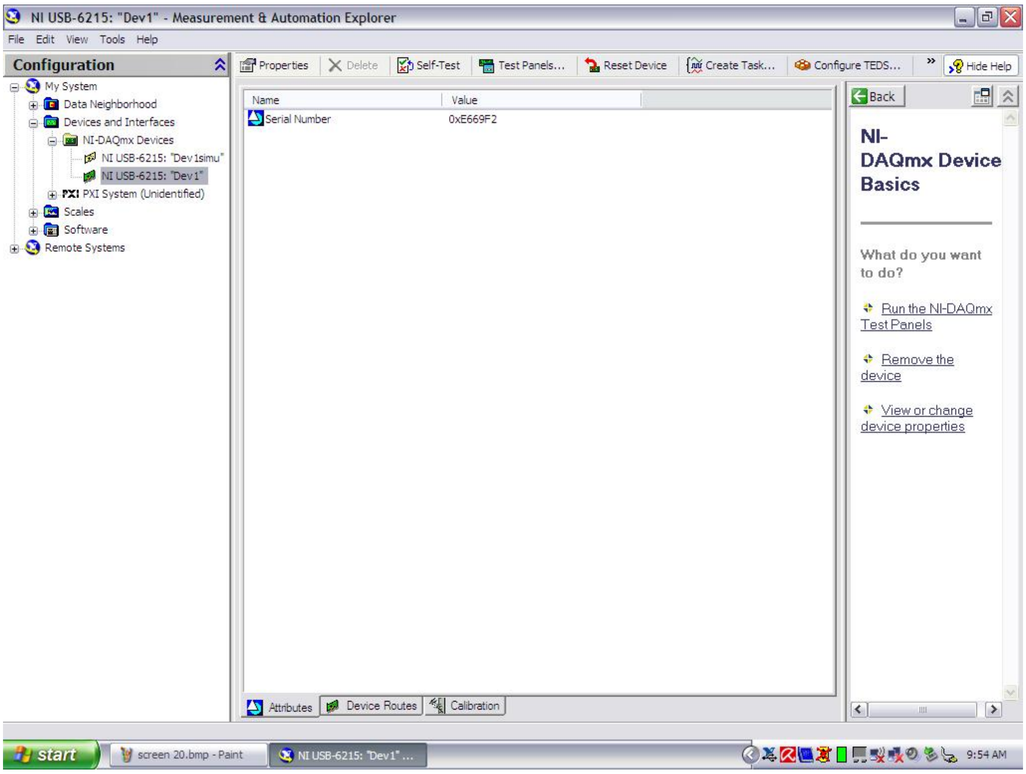
Right click with mouse on the “dev1”, and choose rename, or press F2. Change the name to something different than “dev1”. In this example the name is changed to “dev1simu”, see Screen 30.

Screen 30



Now change the device name of the NI USB-6215 DAQ card to “dev1”. The DAQ-1 instrument should now work properly with AutoResp™ 1.

Screen 31



6.1.2. Another USB device was improperly removed

If an USB device, like a USB memory stick, is not properly removed, while AutoResp™ 1 is running, there can be a connection problem with the DAQ-1 instrument. To solve this problem, close AutoResp™ 1. Then disconnect the USB cable between the PC and the DAQ-1 instrument. Wait 30 seconds. Then connect the cable and wait. When the green diode on the front side of the DAQ-1 instrument is flashing, the connection is re-established. Choose Continue experiment to avoid loosing data.

6.2 Relays are not working

Make sure the DAQ-1 instrument is connected with a power cable, and the Power button is ON.

7. Index

8. Appendix

8.1 pO_2 kPa table

File: PO2 kPa.xls

Partial pressure of oxygen (pO2) at different barometric pressures and temperatures = ((Pbp-Pvap)*.2094)

Temperature (deg C)	0	2	4	6	8	10	12	14	16	18	20	25	30	35	37	40
Pvap (kPa)	0,61	0,71	0,81	0,93	1,07	1,23	1,40	1,60	1,82	2,06	2,34	3,17	4,24	5,62	6,28	7,38
Pbp (kPa)																
97,32	20,256	20,237	20,215	20,191	20,163	20,132	20,097	20,058	20,013	19,964	19,909	19,742	19,526	19,248	19,117	18,895
97,59	20,312	20,292	20,271	20,246	20,218	20,187	20,152	20,113	20,068	20,019	19,963	19,796	19,579	19,301	19,169	18,947
97,85	20,367	20,348	20,326	20,302	20,274	20,242	20,207	20,167	20,123	20,073	20,018	19,850	19,633	19,353	19,221	18,999
98,12	20,423	20,403	20,382	20,357	20,329	20,298	20,262	20,222	20,178	20,128	20,072	19,904	19,686	19,406	19,274	19,050
98,39	20,478	20,459	20,437	20,412	20,384	20,353	20,317	20,277	20,233	20,183	20,127	19,958	19,739	19,459	19,326	19,102
98,65	20,534	20,514	20,492	20,467	20,439	20,408	20,372	20,332	20,288	20,237	20,182	20,012	19,793	19,512	19,378	19,154
98,92	20,589	20,570	20,548	20,523	20,495	20,463	20,427	20,387	20,342	20,292	20,236	20,066	19,846	19,564	19,431	19,206
99,19	20,645	20,625	20,603	20,578	20,550	20,518	20,482	20,442	20,397	20,347	20,291	20,121	19,900	19,617	19,483	19,257
99,45	20,700	20,681	20,658	20,633	20,605	20,573	20,537	20,497	20,452	20,402	20,345	20,175	19,953	19,670	19,536	19,309
99,72	20,756	20,736	20,714	20,689	20,660	20,628	20,593	20,552	20,507	20,456	20,400	20,229	20,007	19,722	19,588	19,361
99,99	20,811	20,791	20,769	20,744	20,716	20,684	20,648	20,607	20,562	20,511	20,454	20,283	20,060	19,775	19,640	19,413
100,25	20,866	20,847	20,825	20,799	20,771	20,739	20,703	20,662	20,617	20,566	20,509	20,337	20,114	19,828	19,693	19,464
100,52	20,922	20,902	20,880	20,855	20,826	20,794	20,758	20,717	20,671	20,620	20,563	20,391	20,167	19,881	19,745	19,516
100,79	20,977	20,958	20,935	20,910	20,881	20,849	20,813	20,772	20,726	20,675	20,618	20,445	20,221	19,933	19,797	19,568
101,05	21,033	21,013	20,991	20,965	20,937	20,904	20,868	20,827	20,781	20,730	20,672	20,499	20,274	19,986	19,850	19,620
101,32	21,088	21,069	21,046	21,021	20,992	20,959	20,923	20,882	20,836	20,784	20,727	20,553	20,328	20,039	19,902	19,672
101,59	21,144	21,124	21,102	21,076	21,047	21,015	20,978	20,937	20,891	20,839	20,782	20,607	20,381	20,092	19,955	19,723
101,85	21,199	21,180	21,157	21,131	21,102	21,070	21,033	20,992	20,946	20,894	20,836	20,661	20,435	20,144	20,007	19,775
102,12	21,255	21,235	21,212	21,187	21,158	21,125	21,088	21,047	21,000	20,948	20,891	20,715	20,488	20,197	20,059	19,827
102,39	21,310	21,290	21,268	21,242	21,213	21,180	21,143	21,102	21,055	21,003	20,945	20,770	20,542	20,250	20,112	19,879
102,65	21,366	21,346	21,323	21,297	21,268	21,235	21,198	21,157	21,110	21,058	21,000	20,824	20,595	20,303	20,164	19,930
102,92	21,421	21,401	21,378	21,353	21,323	21,290	21,253	21,212	21,165	21,113	21,054	20,878	20,649	20,355	20,216	19,982
103,19	21,477	21,457	21,434	21,408	21,379	21,346	21,308	21,267	21,220	21,167	21,109	20,932	20,702	20,408	20,269	20,034
103,45	21,532	21,512	21,489	21,463	21,434	21,401	21,363	21,321	21,275	21,222	21,163	20,986	20,756	20,461	20,321	20,086

8.2 Oxygen solubility

Oxygen solubility in mg O2/liter/kPa at different temperatures and salinities

From Green & Carrit (1967). J. Mar. Biol. 25; 140-147.   1 kPa = 7,501 mmHg

Salinity (o/oo)	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
Temperature (deg C)																			
0	0,6976	0,6878	0,6781	0,6685	0,6591	0,6498	0,6406	0,6316	0,6227	0,6139	0,6053	0,5967	0,5883	0,5800	0,5719	0,5638	0,5559	0,5480	0,5403
1	0,6788	0,6694	0,6600	0,6509	0,6418	0,6329	0,6241	0,6154	0,6068	0,5984	0,5900	0,5818	0,5737	0,5657	0,5579	0,5501	0,5424	0,5349	0,5274
2	0,6608	0,6517	0,6428	0,6339	0,6252	0,6166	0,6082	0,5998	0,5916	0,5834	0,5754	0,5675	0,5597	0,5520	0,5444	0,5369	0,5295	0,5223	0,5151
3	0,6436	0,6349	0,6263	0,6178	0,6094	0,6011	0,5929	0,5849	0,5769	0,5691	0,5614	0,5537	0,5462	0,5388	0,5315	0,5243	0,5171	0,5101	0,5032
4	0,6272	0,6188	0,6104	0,6022	0,5942	0,5862	0,5783	0,5706	0,5629	0,5553	0,5479	0,5405	0,5333	0,5261	0,5190	0,5121	0,5052	0,4984	0,4917
5	0,6114	0,6033	0,5953	0,5874	0,5796	0,5719	0,5643	0,5568	0,5494	0,5421	0,5349	0,5278	0,5208	0,5139	0,5071	0,5004	0,4937	0,4872	0,4807
6	0,5963	0,5885	0,5808	0,5731	0,5656	0,5582	0,5508	0,5436	0,5365	0,5294	0,5225	0,5156	0,5089	0,5022	0,4956	0,4891	0,4827	0,4763	0,4701
7	0,5818	0,5743	0,5668	0,5595	0,5522	0,5450	0,5379	0,5310	0,5241	0,5172	0,5105	0,5039	0,4974	0,4909	0,4845	0,4782	0,4720	0,4659	0,4598
8	0,5680	0,5607	0,5535	0,5463	0,5393	0,5324	0,5255	0,5188	0,5121	0,5055	0,4990	0,4926	0,4863	0,4800	0,4739	0,4678	0,4618	0,4558	0,4500
9	0,5547	0,5476	0,5406	0,5338	0,5270	0,5203	0,5136	0,5071	0,5006	0,4943	0,4880	0,4818	0,4756	0,4696	0,4636	0,4577	0,4519	0,4461	0,4405
10	0,5419	0,5351	0,5283	0,5217	0,5151	0,5086	0,5022	0,4959	0,4896	0,4834	0,4774	0,4713	0,4654	0,4595	0,4537	0,4480	0,4424	0,4368	0,4313
11	0,5297	0,5231	0,5165	0,5101	0,5037	0,4974	0,4912	0,4851	0,4790	0,4730	0,4671	0,4613	0,4555	0,4498	0,4442	0,4387	0,4332	0,4278	0,4224
12	0,5179	0,5115	0,5052	0,4989	0,4928	0,4867	0,4806	0,4747	0,4688	0,4630	0,4573	0,4516	0,4460	0,4405	0,4351	0,4297	0,4244	0,4191	0,4139
13	0,5067	0,5005	0,4943	0,4882	0,4823	0,4763	0,4705	0,4647	0,4590	0,4534	0,4478	0,4423	0,4369	0,4315	0,4262	0,4210	0,4158	0,4107	0,4057
14	0,4959	0,4898	0,4839	0,4780	0,4721	0,4664	0,4607	0,4551	0,4496	0,4441	0,4387	0,4333	0,4281	0,4229	0,4177	0,4126	0,4076	0,4026	0,3977
15	0,4855	0,4796	0,4738	0,4681	0,4624	0,4568	0,4513	0,4459	0,4405	0,4352	0,4299	0,4247	0,4196	0,4145	0,4095	0,4046	0,3997	0,3948	0,3901
16	0,4755	0,4698	0,4641	0,4586	0,4531	0,4476	0,4423	0,4370	0,4317	0,4266	0,4214	0,4164	0,4114	0,4065	0,4016	0,3968	0,3920	0,3873	0,3827
17	0,4659	0,4603	0,4549	0,4494	0,4441	0,4388	0,4336	0,4284	0,4233	0,4183	0,4133	0,4084	0,4035	0,3987	0,3940	0,3893	0,3846	0,3800	0,3755
18	0,4567	0,4513	0,4459	0,4407	0,4354	0,4303	0,4252	0,4202	0,4152	0,4103	0,4054	0,4006	0,3959	0,3912	0,3866	0,3820	0,3775	0,3730	0,3686
19	0,4478	0,4426	0,4374	0,4322	0,4271	0,4221	0,4171	0,4122	0,4074	0,4026	0,3979	0,3932	0,3886	0,3840	0,3795	0,3750	0,3706	0,3662	0,3619
20	0,4393	0,4342	0,4291	0,4241	0,4191	0,4142	0,4094	0,4046	0,3999	0,3952	0,3906	0,3860	0,3815	0,3770	0,3726	0,3683	0,3640	0,3597	0,3555
21	0,4311	0,4261	0,4212	0,4163	0,4114	0,4066	0,4019	0,3972	0,3926	0,3880	0,3835	0,3791	0,3747	0,3703	0,3660	0,3617	0,3575	0,3534	0,3493
22	0,4233	0,4184	0,4135	0,4087	0,4040	0,3993	0,3947	0,3901	0,3856	0,3812	0,3767	0,3724	0,3681	0,3638	0,3596	0,3554	0,3513	0,3473	0,3432
23	0,4157	0,4109	0,4062	0,4015	0,3969	0,3923	0,3878	0,3833	0,3789	0,3745	0,3702	0,3659	0,3617	0,3575	0,3534	0,3494	0,3453	0,3413	0,3374
24	0,4084	0,4037	0,3991	0,3945	0,3900	0,3855	0,3811	0,3767	0,3724	0,3681	0,3639	0,3597	0,3556	0,3515	0,3475	0,3435	0,3395	0,3356	0,3318
25	0,4014	0,3968	0,3923	0,3878	0,3833	0,3790	0,3746	0,3703	0,3661	0,3619	0,3578	0,3537	0,3497	0,3457	0,3417	0,3378	0,3339	0,3301	0,3263
26	0,3947	0,3902	0,3857	0,3813	0,3770	0,3727	0,3684	0,3642	0,3601	0,3560	0,3519	0,3479	0,3439	0,3400	0,3361	0,3323	0,3285	0,3248	0,3211
27	0,3882	0,3838	0,3794	0,3751	0,3708	0,3666	0,3624	0,3583	0,3542	0,3502	0,3462	0,3423	0,3384	0,3346	0,3308	0,3270	0,3233	0,3196	0,3160
28	0,3819	0,3776	0,3733	0,3691	0,3649	0,3608	0,3567	0,3526	0,3486	0,3447	0,3408	0,3369	0,3331	0,3293	0,3256	0,3219	0,3182	0,3146	0,3110
29	0,3759	0,3717	0,3674	0,3633	0,3592	0,3551	0,3511	0,3471	0,3432	0,3393	0,3355	0,3317	0,3279	0,3242	0,3205	0,3169	0,3133	0,3098	0,3063
30	0,3701	0,3659	0,3618	0,3577	0,3537	0,3497	0,3457	0,3418	0,3380	0,3341	0,3304	0,3266	0,3229	0,3193	0,3157	0,3121	0,3086	0,3051	0,3017

Constants:

-7,424	-0,1288	273,16
4417	53,44	
-2,927	-0,0444	
0,04238	0,00071	

John Fleng Steffensen, 2002

8.3 DAQ-1 Instruction manual

The DAQ-1 instrument is used for data acquisition and relay controlling. It is designed to run with the software AutoResp™ 1, but it can be used with other applications.

LIST OF PARTS
DAQ-1 instrument
AutoResp™ 1
Power Cord
USB cable
Adapter cable for pumps, qty. 2
User manual

SETUP
To power up the instrument connect the power cord to the outlet 100-240 VAC 50-60Hz on the back side of the instrument. Connect the USB cable to an USB port on your PC and to the outlet on the front side of the instrument named PC.

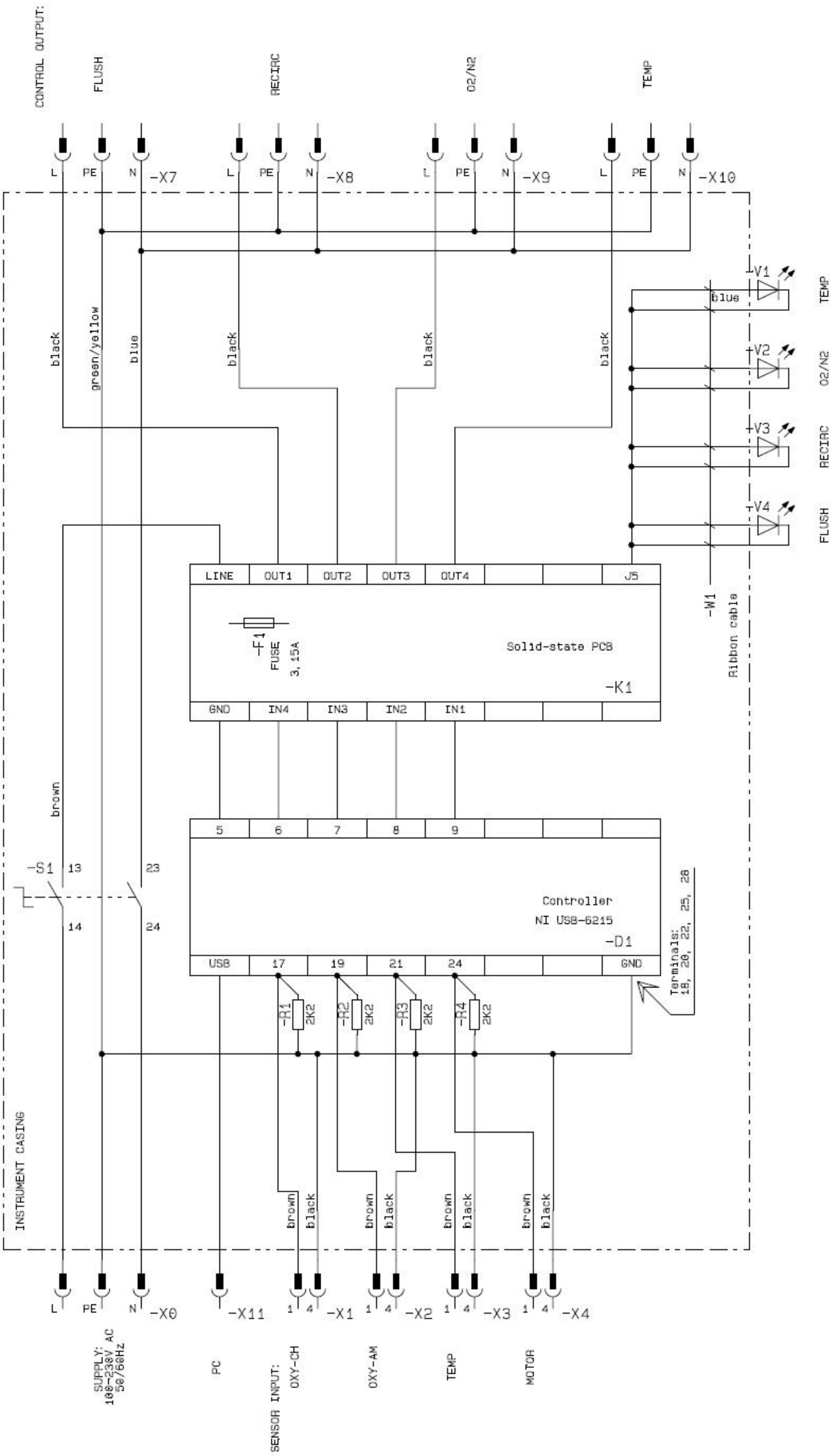
USING THE DAQ-1 FOR DATA ACQUISITION
The DAQ-1 instrument acquires data on 4 channels and converts the analog signals to 16 bit values. Connect the inputs to controller instruments, e.g. OXY-AM to an OXY-REG with a data cable. If you want to use controller instruments not from Loligo Systems make sure the input range is 0-5 VDC. The signal must be on pin 1 and GND on pin 4 on an input connector.

Using the DAQ-1 in other applications
It is possible to use the DAQ-1 instrument with other applications than AutoResp™ 1. See Table 3 for input connections and the wiring diagram on Figur 1.

Table 3

DAQ-1 connector	NI-USB 6215 pins	Channel name
OXY-CH	17	Ai1
OXY-AM	19	Ai2
TEMP	21	Ai3
MOTOR	24	Ai4
GND	18, 20, 22, 25 and 28	n.A.

Figur 1


CONTROL OUTPUT:
FLUSH
RECIRC
O2/N2
TEMP
-X7
-X8
-X9
-X10
black
green/yellow
blue
brown
blue
TEMP
O2/N2
RECIRC
FLUSH
LINE
OUT1
OUT2
OUT3
OUT4
J5
-F1
FUSE
3,15A
Solid-state PCB
-K1
GND
IN4
IN3
IN2
IN1
-W1
Ribbon cable
Controller
NI USB-6215
-D1
USB
GND
Terminals:
18, 20, 22, 25, 28
-S1
2K2
INSTRUMENT CASING
-X0
-X11
-X1
-X2
-X3
-X4
SUPPLY:
100-230V AC
50/60Hz
PC
SENSOR INPUT:
OXY-CH
OXY-AM
TEMP
MOTOR

For using the LEDs and the relays see Table 4 and the wiring diagram on Figur 1.

Table 4

DAQ-1 connector	NI-USB 6215 pins	Channel name line1
FLUSH	6	D0
RECIRC	7	D1
O_2/N_2	8	D2
TEMP	9	D3
GND	5	n.A.

Through the Solid state PCB in the DAQ-1 instrument it is possible to connect up to 500 W equipment to every relay.

SPECIFICATIONS

Specifications range

-20°C to +60°C

Common specifications

Supply voltage (universal): 21.6-253 VAC, 50-60 Hz or 19.2-300 VDC
Internal consumption: 3.2 W
Max. consumption: 3.5 W
Isolation voltage (test / operation): 2.3 kVAC / 250 VAC
Signal- / noise ratio: Min. 60 dB (0-100 kHz)
Response time, programmable: 0.4-60 s
Calibration temperature: 20-28°C
Accuracy: ≤±0.1% of reading
Temperature Coefficient: ≤±0.01% of reading/ °C
EMC immunity influence: ≤±0.5% of reading
Potentiometer input, min: 10 Ω
Potentiometer input, max: 100 kΩ

Relay outputs

Relay function: Setpoint
Hysteresis, in % / display counts: 0.1-25% / 1-2999
On and Off delay: 0-3600 s
Sensor error detection: Make / Break / Hold
Max. voltage: 250 VRMS
Max. current: 2 A / AC
Max. AC power: 500 VA
Max. current at 24 VDC: 1 A

8.4 Fiber optic oxygen instrument instruction manual

The instruction manual for the fiber optic instrument from PreSens is included as an individual document. Please use this manual for further information about the fiber optic oxygen instrument and the software. Please look into the DAQ-PAC-F1S package

Instruction Manual

FIBOX 3

Fiber-optic oxygen meter

Instruction Manual
Fibox 3

Software Version 5.32

March 2006

Specification of Fibox 3:

PC-controlled one-channel fiber-optic oxygen meter for oxygen minisensors;

excitation wavelength of 505 nm;

polymer optical fibers (POF) of 2 mm diameter connected by SMA fiber connectors.

Manufacturer

PreSens
Precision Sensing GmbH
Josef-Engert-Straße 9
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Internet	www.presens.de

Table of Contents

1 Preface

Congratulations!

You have chosen a new innovative technology for measuring oxygen!

The Fibox 3 is a compact, easy to transport and completely PC-controlled fiber-optic oxygen meter. The data evaluation is PC-supported as well.

The Fibox 3 was specially developed for small fiber-optic oxygen sensors, flow-trough cells and integrated sensor systems. It is based on a novel technology, which creates very stable, internally referenced, measured values. This enables a more flexible use of oxygen sensors in many different fields of interest.

Optical oxygen sensors (also called optodes) have the following outstanding properties:

- They are small
- They do not consume oxygen
- Limit of detection up to 15 ppb
- On-line, **non-invasive** and **non-destructive** oxygen measurements
- They can measure in both liquids as well as gas phase (headspace)
- Their signal does not depend on the flow rate of the sample
- They have excellent long-term stability
- They can be physically divided from the measuring system which means a contactless measurement
- They can be autoclaved and γ-sterilized

Therefore, they are ideally suited for the examination of small sample volumes, long-term measurements in difficult samples, and for biotechnological applications.

A set of different oxygen minisensors, flow-through cells and integrated sensor systems is available to make sure you have the sensor which is ideally suited to your application.

Please feel free to contact our service team to find the best solution for your application.

Your PreSens Team

2 Safety Guidelines

PLEASE READ THESE INSTRUCTIONS CAREFULLY BEFORE WORKING WITH THIS INSTRUMENT!

This device has left our works after careful testing of all functions and safety requirements.

The perfect functioning and operational safety of the instrument can only be ensured if the user observes the usual safety precautions as well as the specific safety guidelines stated in these operating guidelines.

- Before connecting the device to the electrical supply network, please ensure that the operating voltage stated on the power supply corresponds to the mains voltage.
- The perfect functioning and operational safety of the instrument can only be maintained under the climatic conditions specified in Chapter 10 "Technical Data" in this operating manual.
- If the instrument is moved from cold to warm surroundings, condensate may form and interfere with the functioning of the instrument. In this event, wait until the temperature of the instrument reaches room temperature before putting the instrument back into operation.
- Balancing, maintenance and repair work must only be carried out by a suitable, qualified technician, trained by us.
- Especially in the case of any damage to current-carrying parts, such as the power supply cable or the power supply itself, the device must be taken out of operation and protected against being put back into operation.
- If there is any reason to assume that the instrument can no longer be employed without a risk, it must be set aside and appropriately marked to prevent further use.
- The safety of the user may be endangered, e. g., if the instrument
    - is visibly damaged
    - no longer operates as specified
    - has been stored under adverse conditions for a lengthy period of time
    - has been damaged in transport
- If you are in doubt, the instrument should be sent back to the manufacturer PreSens for repair and maintenance.
- The operator of this measuring instrument must ensure that the following laws and guidelines are observed when using dangerous substances:
    - EEC directives for protective labor legislation
    - National protective labor legislation
    - Safety regulations for accident prevention
    - Safety data-sheets of the chemical manufacturer

The Fibox 3 is not protected against water spray
The Fibox 3 is not water-proof
The Fibox 3 must not be used under environmental conditions which cause water condensation in the housing
The Fibox 3 is sealed
The Fibox 3 must not be opened

We explicitly draw your attention to the fact that any damage of the manufactural seal will render of all guarantee warranties invalid.

Any internal operations on the unit must be carried out by personal explicitly authorized by PreSens and under antistatic conditions.

The Fibox 3 may only be operated by qualified personal.

This measuring instrument was developed for use in the laboratory. Thus, we must assume that, as a result of their professional training and experience, the operators will know the necessary safety precautions to take when handling chemicals.

Keep the Fibox 3 and the equipment such as PT 100 temperature sensor, power supply and optical sensors out of the reach of children!

As the manufacturer of the Fibox 3, we only consider ourselves responsible for safety and performance of the device if

- the device is strictly used according to the instruction manual and the safety guidelines
- the electrical installation of the respective room corresponds to the DIN IEC/VDE standards.

The Fibox 3 and the sensors must not be used for in-vivo examinations on humans!

The Fibox 3 and the sensors must not be used for human diagnostic or therapeutical purposes!

3 Description of the Fibox 3 Device

The **Fibox 3** is a precision, ***temperature-compensated*** oxygen meter designed for fiber-optic oxygen minisensors.

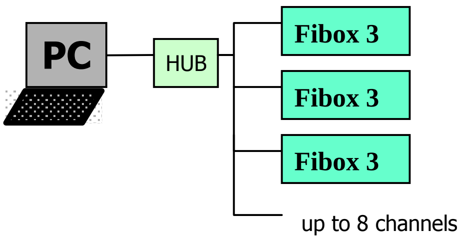
Its robust design and low power consumption makes it ready for indoor and **outdoor** application.

For operation, a PC/Notebook with RS232 interface is required. The Fibox 3 is controlled using a comfortable software, which also saves and visualizes the measured values.

Fibox 3 instrument features:

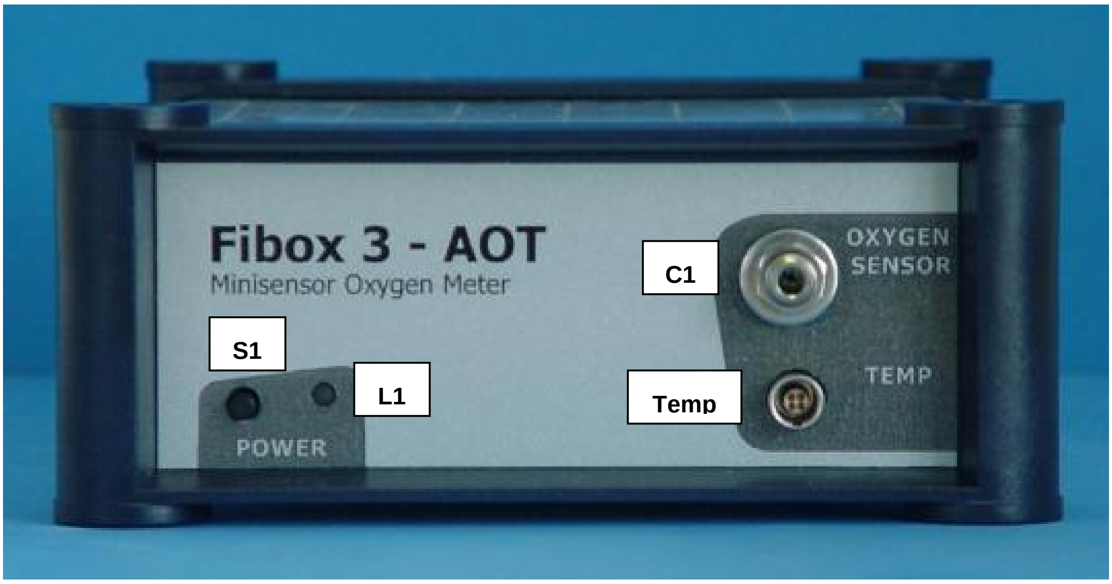
- high precision
- portable (battery power optional)
- analog/digital data output (on request)
- temperature compensation

It is also possible to combine several single Fibox 3 oxygen meters to obtain a multichannel system. It allows the user to create a 2, 3, 4 or more channel system.



The Fibox 3 oxygen meter contains a dual 12 bit analog output and an external trigger input. The analog output values can be programmed with the PC software (included). The user can choose between oxygen, temperature, amplitude or phase for each channel independently. The Fibox 3 can be used as a stand-alone instrument when combined with an external data logger.

Front Panel of the Fibox 3 device

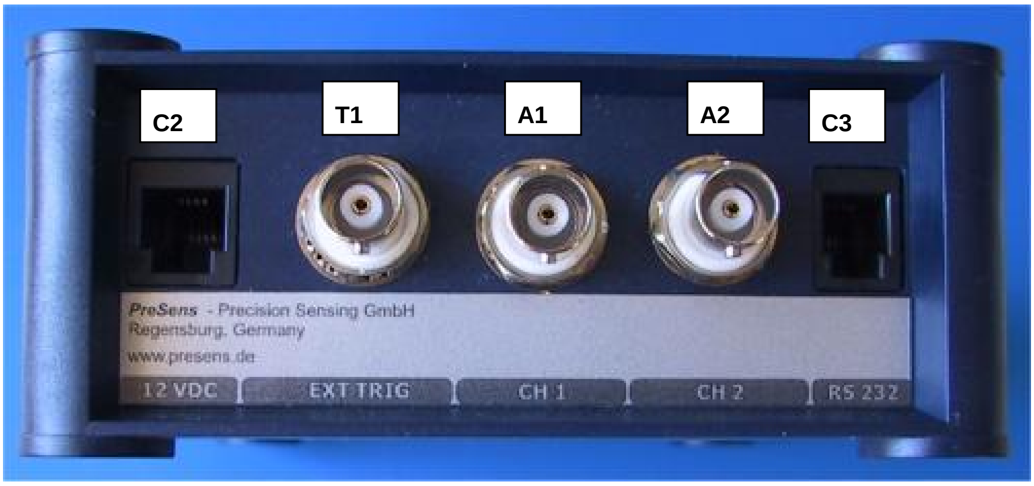


ELEMENT	DESCRIPTION	FUNCTION
S1	ON/OFF switch	Switches the device ON and OFF
C1	SMA fiber connector	Connect the fiber-optic oxygen minisensor here.
L1	Control LED	red: instrument off green: instrument on orange: stand by
T1	Connector for PT 1000 temperature sensor	Connect the PT 1000 temperature sensor for temperature-compensated measurements here.

Rear Panel of the Fibox 3 device

Fibox 3 has two standard BNC connectors (A1, A2) for analog output channels 1 and 2, and another one (T1) for external trigger input (see figure below).

The electrical specifications of all rear panel connectors are given in the technical specification sheet. Please read also the technical notes to avoid mistakes.



The electrical specification of all rear panel connectors is given in the technical specification sheet. Please read also the technical notes to avoid mistakes.

ELEMENT	DESCRPTION	FUNCTION
C2	Line adapter for power supply	Connector for 9 - 36 V DC power supply.
C3	RS232 interface (male)	Connect the device with a RS232 data cable to your PC/Notebook here.
A1	Analog out (channel 1)	Connect the device with external devices, e.g. a data logger
A2	Analog out (channel 2)	Connect the device with external devices, e.g. a data logger
T1	External trigger input	Connect the device with external devices, e.g. data logger with a trigger output, pulse generator

Features

- minisensor oxygen meter with temperature compensation
- 2 x 12bit, programmable analog channels, with optical isolation
- 9 – 36 V supply voltage (or 220/110V AC adapter)
- RS 232 interface, with optical isolation
- robust metal box

4 Required Basic Equipment

- Oxygen meter Fibox 3*
- Software for Fibox 3*
- PC / Notebook
  (System requirements: Windows 95/98/2000/XP//Millenium/NT 4.0; Pentium processor, at least 133 MHz, 16 MB RAM)
- RS 232 Cable *
- Line adapter (110 - 220 V AC, 12 V DC) *
- Temperature sensor PT 1000*
- Oxygen-sensitive minisensor
  The minisensors are mounted into different types of housings
- Vessels for calibration standard 100 (water vapor-saturated air ≡ 100 % air saturation) and calibration solution 0 (oxygen-free water)

  We recommend Schott laboratory bottles with a thread which can be obtained by Merck Eurolab (ordering number: 215L1515)
- Laboratory support with clamp, micro-manipulator

*: scope of supply

5 Planar Oxygen Minisensors

5.1 Sensor Characteristic of Oxygen-Sensitive Minisensors

The principle of the sensor operation is based on the quenching of luminescence caused by collision between molecular oxygen and luminescent dye molecules in the excited state. Figure 5.1 shows a typical response curve of an oxygen-sensitive sensor. In the presence of oxygen the signal - in our case the phase angle Φ - decreases. The phase angle Φ can be related to the oxygen content as shown in Figure 5.2. The theoretical aspects are explained more detailed in the appendix.

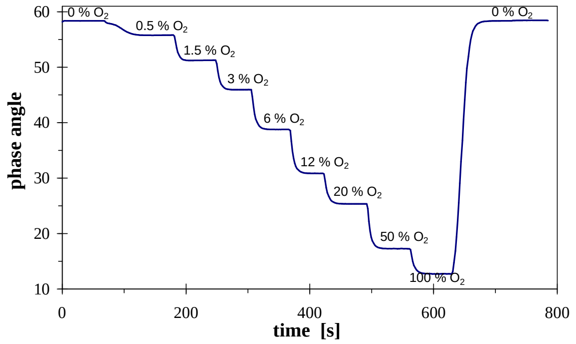


Figure 5.1 Response of minisensor **PSt3** toward changes in the oxygen concentration.

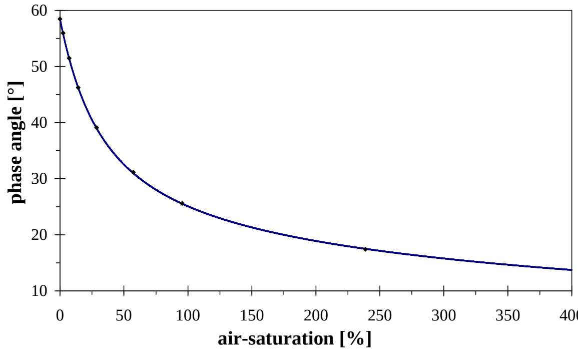


Figure 5.2 Effect of the phase angle of minisensor **PSt3** on different oxygen contents.

Measuring range

PreSens offers an oxygen sensor membrane called **PSt3** which is tailored for oxygen measurements up to 250 % air-saturation. The measuring range and the limit of detection of this sensor are given in Table 5.1.

Table 5.1 *Measuring range and limit of detection of the* ***PSt3 oxygen sensor membrane****.*

	Dissolved Oxygen	Gaseous & Dissolved Oxygen
Measurement range	0 - 22 mg/L (ppm) 0 - 700 µmol	0 - 250 % air-sat. 0 - 50 % oxygen-sat. 0 - 380 Torr 0 - 500 hPa
Limit of Detection (LOD)	0.15 % air-saturation, 15 ppb dissolved oxygen	0.31 hPa, 0.23 Torr

Resolution and accuracy

The PreSens Fibox 3 has a phase resolution of smaller than 0.05°. Since the oxygen calibration plot displays a non-linear behavior, the oxygen resolution is given for four different partial pressures at 20 °C, the accuracy for two different partial pressures. The resolution in oxygen is also transformed in different oxygen units.

Table 5.2. *Oxygen resolution and accuracy of the **PSt3 oxygen sensor membrane** at different oxygen contents at 20 °C and 1013 mbar.*

	Dissolved Oxygen	**Gaseous & Dissolved Oxygen**
Resolution **at 20 °C and 1013 hPa**	0.09 ± 0.005 mg/L (ppm) 2.72 ± 0.01 mg/L (ppm) 9.06 ± 0.05 mg/L (ppm) 22.65 ± 0.15 mg/L (ppm)	1 ± 0.05 % air-sat. 30 ± 0.1 % air-sat. 100 ± 0.5 % air-sat. 250 ± 1.7 % air-sat.
		0.21 ± 0.01 % oxygen 6.3 ± 0.02 % oxygen 20.9 ± 0.1 % oxygen 52.4 ± 0.35 % oxygen
	2.83 ± 0.14 µmol 85.0 ± 0.28 µmol 283.1 ± 1.4 µmol 798.0 ± 4.7 µmol	1.55 ± 0.08 Torr 46.7 ± 0.2 Torr 155.5 ± 0.75 Torr 388.8 ± 2.6 Torr
		2 ± 0.1 hPa 60 ± 0.3 hPa 200 ± 1 hPa 500 ± 0.3 hPa
Accuracy (20 °C)	± 1% at 100 % air-saturation; ± 0.15% at 1 % air-saturation	

Temperature

PreSens oxygen sensors can be used in the temperature range of -10 to 50 °C. PreSens offers a PT 1000 temperature sensor in combination with the Fibox 3 to record temperature variations which are compensated using the Fibox 3 software (see Chapter 7, ***Calibration*** and Chapter 8, ***Measurement***).

Cross sensitivity:

No cross sensitivity exists for ***carbon dioxide*** (CO_2), ***hydrogen sulfide*** (H_2S), ***ammonia*** (NH_3), ***pH***, any ionic species like ***sulfide*** (S_2^-), ***sulfate*** (SO_4^{2-}), ***chloride*** (Cl^-) or ***salinity***. Turbidity and changes in the stirring rate have no influence on the measurement.

The sensors can also be used in ***methanol***- and ***ethanol-water*** mixtures as well as in ***pure methanol*** and ***ethanol***.

We recommend to avoid other organic solvents, such as acetone, chloroform or methylene chloride, which may swell the sensor matrix.

Interferences were found for gaseous sulfur dioxide (SO_2) and gaseous chlorine (Cl_2). Both of them mimic higher oxygen concentrations.

Response time

The response time (t_{90}, 90 % of the signal change has occurred) of the **PSt3** oxygen sensor is less than 20 s in solution (stirred) and even less than 6 s in the gas phase.

The response time (t_{90}) of the oxygen sensor is dependent from the diffusion rate of oxygen through the sensor layer, and, hence, on the thickness of the sensor layer and the stirring rate. A typical oxygen response curve of sensor membrane **PSt3** in a non-stirred and stirred sample solution is shown in Figure 5.3. The response times (t_{90}) of sensor membrane **PSt3** are listed in Table 5.3.

Unlike electrodes, optical sensors do **not** consume oxygen and the signal is independent of changes in flow velocity, which means that stirring decreases the response time, but has no effect on the measured value.

Optical isolation of the oxygen-sensitive layer which is applied to exclude ambient light and improve chemical resistance will slow down the sensor response.

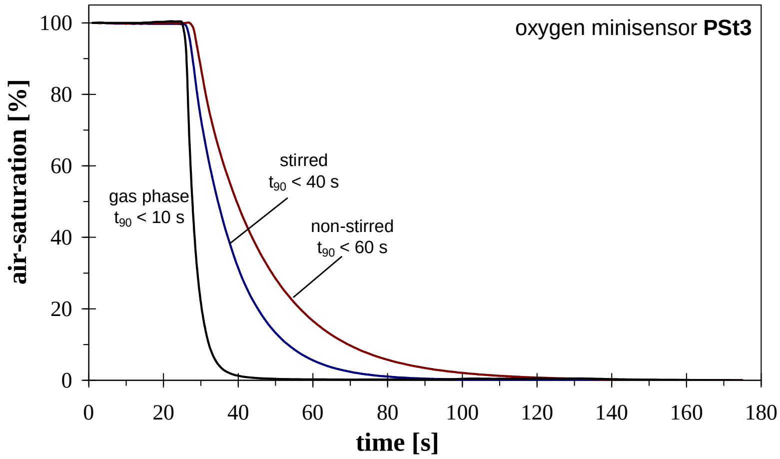


Figure 5.3 Response characteristic of an optical isolated oxygen sensor **PSt3** in a stirred, a non-stirred sample solution and in the gas phase.

Table 5.3 Response time (t_{90}) of PreSens oxygen sensors.

	dissolved oxygen		**gaseous oxygen**
	stirred	**not stirred**	
oxygen sensor PSt3			
t_{90} without optical isolation	< 20 s	< 40 s	< 6 s
t_{90} with optical isolation	< 30 s	< 60 s	< 10 s

Optical isolation

Optical isolated sensor tips are required if your sample shows intrinsic fluorescence between 540 - 700 nm. Consequently, an optical isolation is recommended measuring in whole blood, urine or chlorophyll-containing samples. Using optical isolated sensors excludes the impact of colored samples and ambient light on measurements. Furthermore, the optical isolation layer is applied to exclude strong ambient light, to improve chemical resistance especially against oily samples as well as to reduce bio-fouling on the sensor membrane.

Optical isolated sensor tips of oxygen sensors enable measurement in photosynthetically active samples, since stimulation of photosynthesis due to emission of blue-green light from the fiber tip is avoided.

PreSens offers additional optical isolation for all types of oxygen sensors.

Sensor Stability

The oxygen-sensitive membrane stands gamma-sterilization, sterilization by ethylene oxide, steam autoclavation (140 °C, 1.5 atm), CIP conditions (cleaning-in-place, 5 % NaOH, 90 °C), as well as a 3 % H_2O_2 solution.

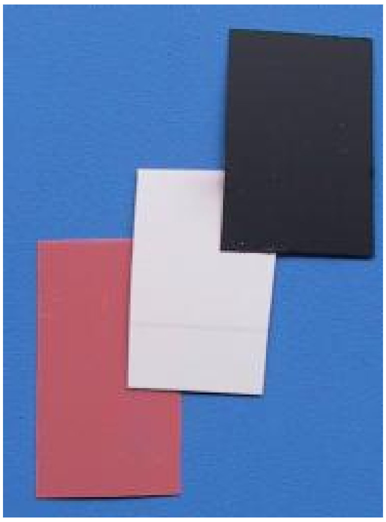
The oxygen-sensitive material may be subject to photodecomposition resulting in a signal drift. Photodecomposition takes place only during illumination of the sensor tip and depends on the intensity of the excitation light.

Table 5.4. *Drift in % air-saturation at 100 % air-saturation when illuminating the oxygen sensor **PSt3** for 1, 12 and 24 hours in the continuous mode (1 sec mode).*

	Drift per hour	**Drift per 12 hours**	**Drift per 24 hours**
PSt3	---	---	< 0.4 % air-saturation

5.2 Housings of Oxygen-Sensitive Minisensors

PreSens fiber-optic oxygen sensors are based on 2 mm polymer optical fibers (**POF**). Depending on the respective application, PreSens offers a set of different standard designs.



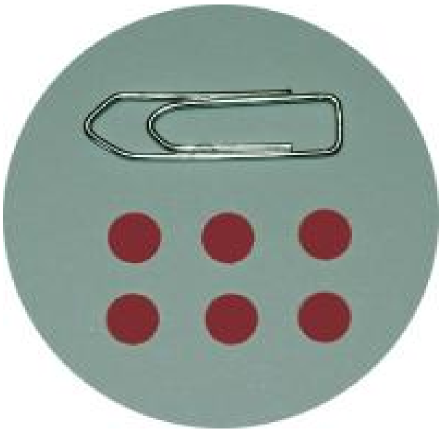
Planar oxygen-sensitive **sensor foils (SP)**

Flow-through cell design connected to a 2 mm POF **(FTC)**

2 mm **Dipping Probe (DP)** with coated sensor foil

Of course, it is possible to build ***customer-specific*** designs. Please feel free to contact our service team to find the best solution for your application.

5.2.1 Planar Oxygen-Sensitive Foils (SP-PSt3)

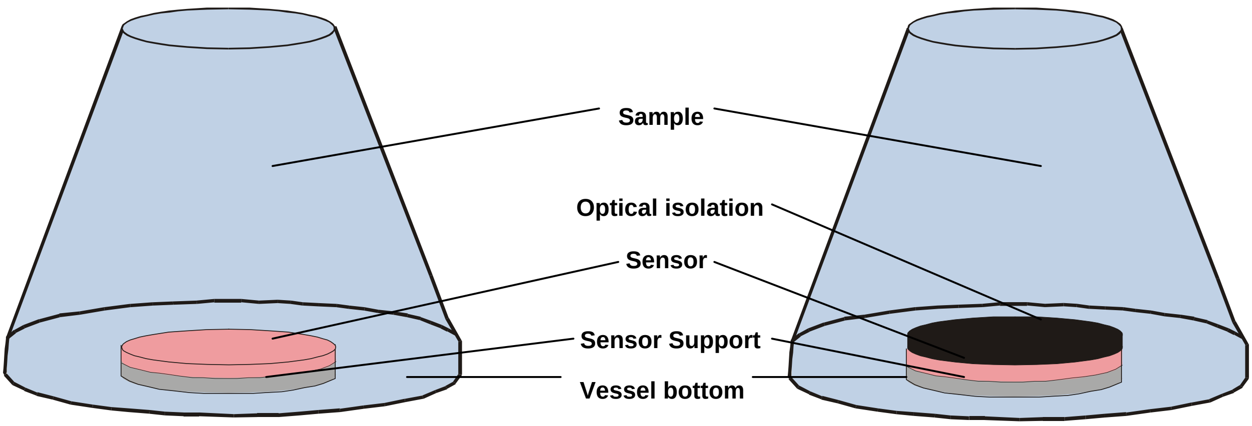


Planar oxygen sensors **SP-PSt3** immobilized onto different supports (polyester, glass) are available for customer- specific applications. Sensors based on a polyester support can be easily cut into small pieces using a razor blade. Round spots (sensor spots) of 3 mm in diameter can be punched.

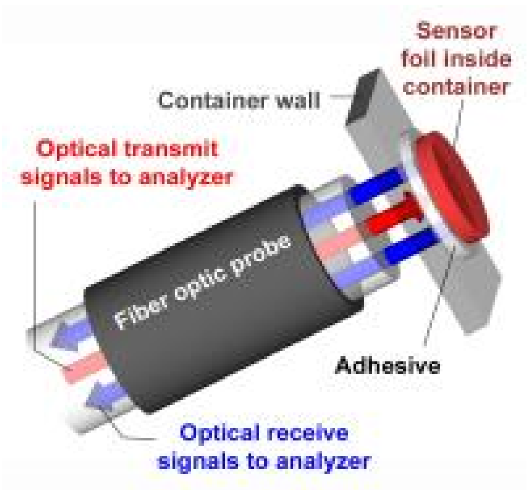
The **sensor spots** can be glued, for example, inside glass vials such as cell culture flasks, bags, and disposables. The oxygen concentration can be measured **non-invasively** and **non-destructively** from outside through the wall.

Only prerequisite: The wall has to be transparent and non-fluorescent.

Please note: Be sure to glue the sensor spots onto your vessel with the proper side! The sensor support (polyester foil or glass) on which the sensor is spotted (identifiable by its faint reflection) is glued to the vessel, while the sensor itself must look toward the sample. The figure below shows how the (highly enlarged) sensor spot must be glued to the vessel.



Sensor spot (SP) glued into a vessel (no optical isolation). Optical isolated sensor spot (SP) glued into a vessel.



Features

- non-invasive and non-destructive measurement from outside through the wall of the flask
- excellent mechanical stability and long-term stability (more than 100000 data points without drift)
- online monitoring
- response time (t_{90}) in the order of 30 s
- measuring range: 0-250 % a.s.
- limit of detection: 0.15 % a.s.

Oxygen sensor immobilized onto a glass support

- stands CIP (Cleaning In Place) conditions
- sterilizable (autoclave (130 °C, 1.5 atm), ethanol, ethylene oxide, H_2O_2)

Oxygen sensor immobilized onto a polyester support

- stands CIP (Cleaning In Place) conditions
- sterilizable (ethanol, ethylene oxide, H_2O_2)
- **not** autoclavable
- flexible

A polymer optical fiber is used as a light guide between the Fibox oxygen meter and a sensor foil (SP-PSt3) which was glued inside a glass vial to read out the analyte concentration non-invasively and non-destructively **from outside through the transparent wall of the flask**.

Ordering information

SP – PSt3 – [ ] AU – D [ ] – [ ] OP

Oxygen **S**ensor **S**pot

Oxygen-sensitive coating
PSt3: 0-50% oxygen

Autoclavable
- **Y**: Yes
- **N**: No

Spot diameter [mm]
- 5
- 10

Optical isolation
- **Y**: with opt. isolation
- **N**: without opt. isolation

Example

SP – PSt3 – Y AU – D 5 – Y OP

With this code you will order a planar autoclavable (***YAU***) oxygen sensor spots (***SP***), type ***PSt3*** (0 - 50 % oxygen) with optical isolation (***YOP***). The spot diameter is 5 mm (***D5***).

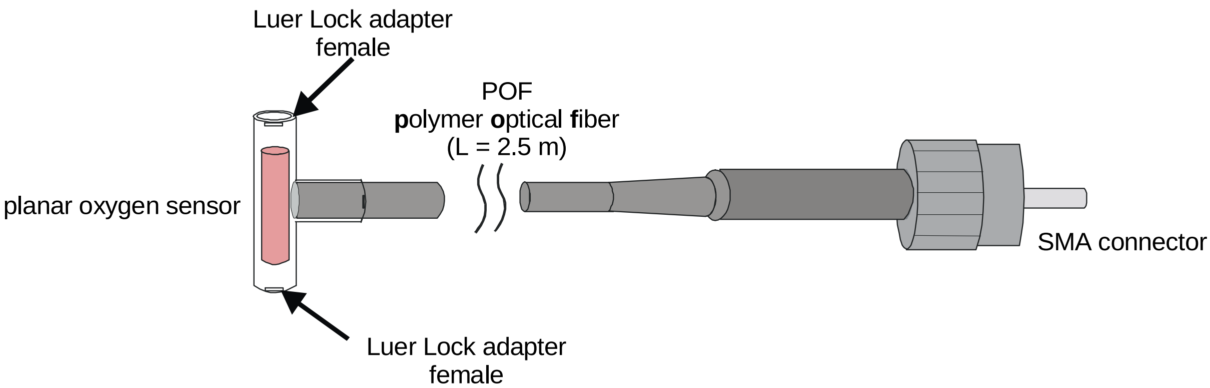
5.2.2 Flow-Through Cell with Integrated Planar Oxygen Sensor (FTC-PSt3)

The flow-through oxygen minisensor **(FTC-PSt3)** is a miniaturized fiber-optic chemical sensor integrated in a T-shape flow-through cell.

The flow-through cell is connected to the Fibox oxygen meter by a polymer optical fiber with 2 mm diameter as a light guide. A glass tube with 2 mm inner diameter (4 mm outer diameter) is coated with oxygen-sensitive dye at its inner wall. The volume for liquid inside the FTC cell is about 100 (± 10) µL.

The standard T-shape flow cell can be easily connected via Luer-Lock adapters to external tubings. Liquids (like water, blood, etc.) can be pumped through the cell.

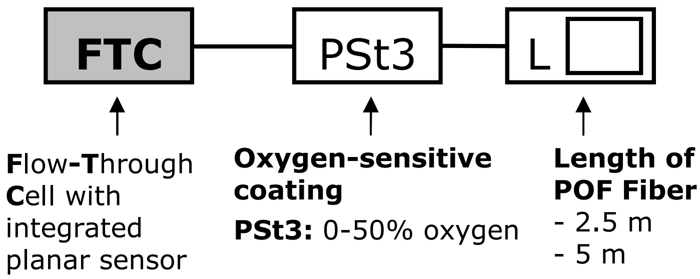
Schematic drawing of flow-through cell oxygen sensors



Features

- very robust sensor with an excellent long-term stability (more than 100000 data points without drift)
- online monitoring
- sterilizable (autoclave (130 °C, 1.5 atm), ethanol, ethylene oxide)
- response time (t_{90}) in the order of 1 minute
- measuring range: 0-250 % a.s.
- limit of detection: 0.15 % a.s.
- stands CIP conditions (cleaning-in-place, 5 % NaOH, 90°C)

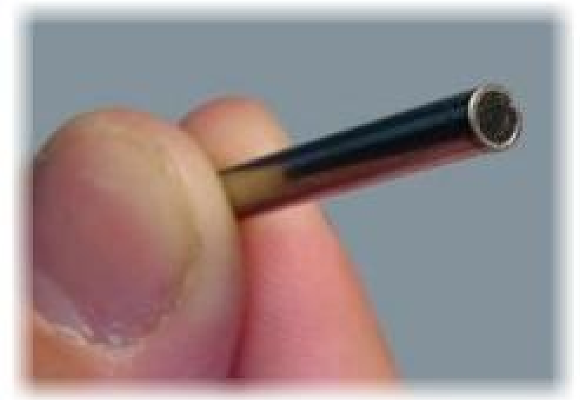
Ordering Information



Example

With **FTC-PSt3-L2.5** you will order a flow-through cell (***FTC***) with sensor type ***PSt3*** (0-50 %oxygen). The standard cable length is 2.5 m (***L2.5***), the maximum length is 15 m.

5.2.3 Oxygen Dipping Probe (DP-PSt3)

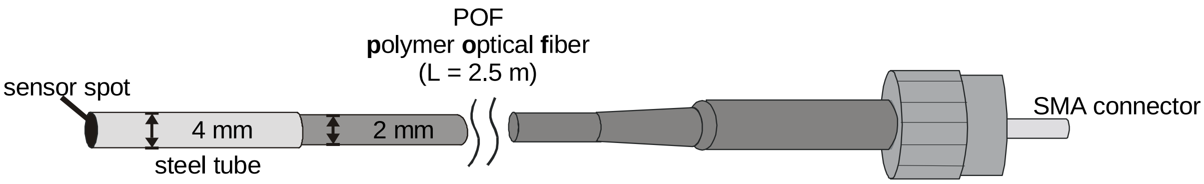


This oxygen sensor consists of a polymer optical fiber with a polished distal tip which is coated with a planar oxygen-sensitive foil.

The end of the polymer optical fiber is covered with a high-grade steel tube, to protect both the sensor material and the POF.

Usually, the fiber is coated with an optical isolated sensor material in order to exclude ambient light from the fiber tip.

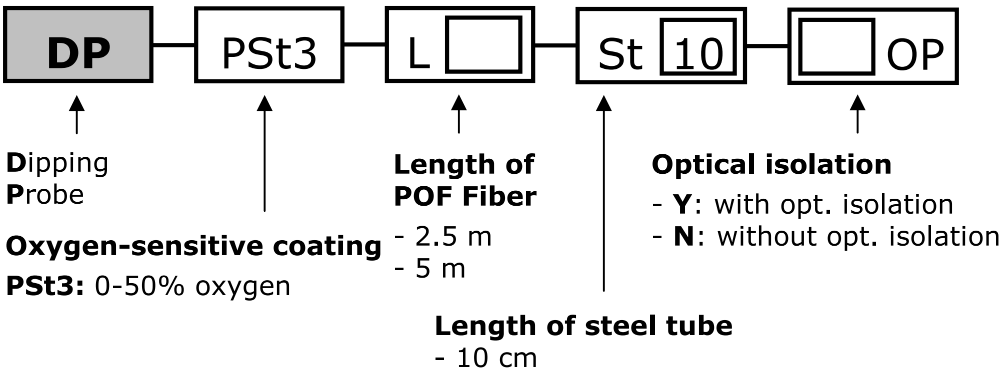
Schematic drawing



Features

- usable for process application
- very robust sensor with an excellent long-term stability (more than 100000 data points without drift)
- sterilizable (H_2O_2, ethanol, ethylene oxide)
- **not** autoclavable (POF does not stand autoclaving conditions (130 °C, 1.5 atm))
- measuring range: 0-250 % a.s.
- limit of detection: 0.15 % a.s.

Ordering information

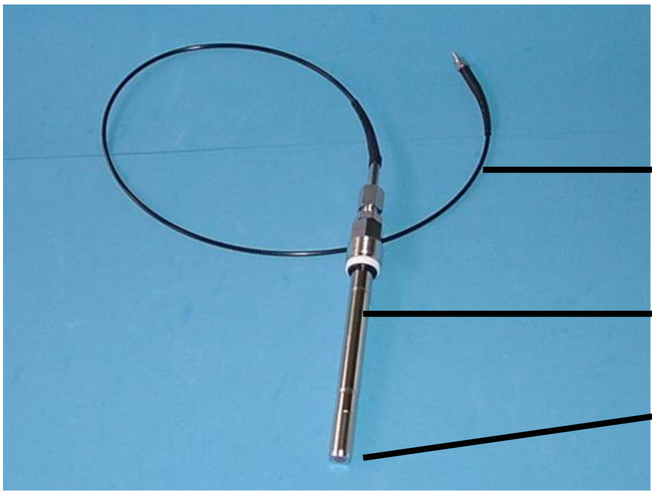


Example

With **DP-PSt3-L2.5-ST10-YOP** you will order a dipping probe (***DP***) coated with an optical isolated (***YOP***) oxygen sensor coating type ***PSt3*** (0 - 50 % oxygen). The standard cable length is 2.5 m (**L2.5**), the maximum length is 25 m. The length of the protection steel tube is 10 cm (**ST10**).

5.2.4 Oxygen Probe for Inline Measurements in Fermenters (OIM)

OIM consists of a fitting made from stainless steel. The oxygen sensor is integrated in the top of the metal fitting (as shown below). The metal fitting is connected to the instrument via a polymer optical fiber. The standard fiber cable length is 2.5 m. OIM is available in different sizes (12 mm, 25 mm) and standard OIM fits to B. Braun Biostat B and B. Braun Biostat C fermenters.



Polymer optical fiber

Steel housing (diameter: 12 mm)

DO Probe immobilized on a glass support

This chemo-optical DO probe has outstanding properties:

- The system can be used after autoclavation without recalibration
- The system is fully autoclavable up to 100 times
- In contrast to classical oxygen electrodes, membrane cleaning and frequent replacement is not necessary
- There are no electrolyte solutions to poison or replenish
- No time for polarization is needed
- Long shelf-life
- measuring range: 0-250 % a.s.
- limit of detection: 0.15 % a.s.

Please note: The OIM is only ready for use together with the Optical Exchange Cap (OEC, see page 17) containing the oxygen sensor membrane.

Ordering information

OIM – **D12 / L** ☐ – **L** ☐

Oxygen **P**robe for **I**nline **M**easurement

Steel tube
Diameter [mm] / Length [mm]
12 / 120
12 / 160
12 / 215
12 / 325

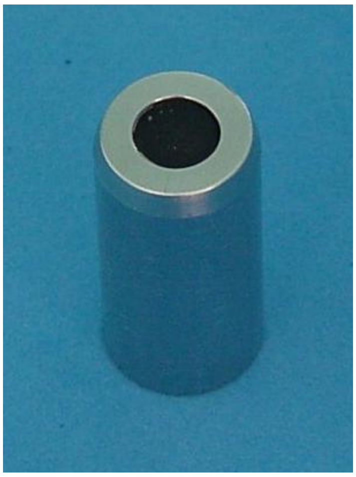
Length of the fiber cable
- **2.5**: 2.5 m
- **5**: 5 m

5.2.5 OIM Exchange Cap (OEC-PSt3)

Applications:

The **O**IM **E**xchange **C**ap (**OEC**) is the sensitive coating in a metal cap. It is used to replace the old sensitive coating and has outstanding properties:

- The PSt3-coated OEC can be used after autoclavation without recalibration
- The PSt3-coated OEC is autoclavable up to 100 times
- In contrast to classical oxygen electrodes, membrane cleaning and frequent replacement is not necessary
- The PSt3-coated OEC can be easily exchanged
- There are no electrolyte solutions to poison or replenish
- No time for polarization is needed
- Long shelf-life
- Optical isolation prevents cross-sensitivity of the sensor towards turbid or fluorescent samples.
- measuring range: 0-250 % a.s.
- limit of detection: 0.15 % a.s.



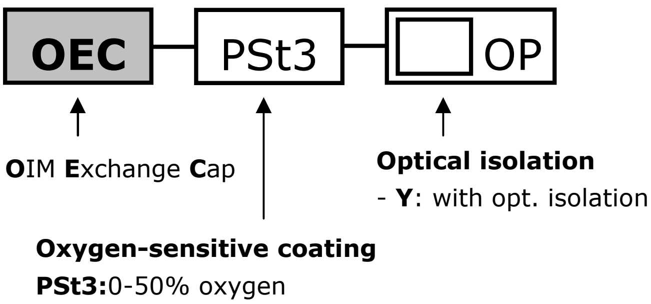
The OEC is coated with the oxygen-sensitive material **PSt3**. It has to be integrated into the Oxygen Probe OIM.

The sensor signal is transmitted to the instrument via a polymer optical fiber. The standard fiber cable length is 2.5 m. If you need a cable length of more than 2.5 m (up to 15 m available), please contact our service team.

Response times of the oxygen sensor OEC-PSt3

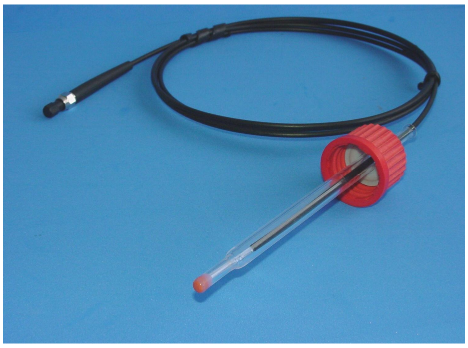
Response time	Stirred	Not stirred	Gaseous Oxygen
t_{90}	< 60 s	< 120 s	< 20 s

Ordering information



Order code for the OIM Exchange Cap: OEC-PSt3-YOP

5.2.6 OxyFinger Chemo-Optical DO Probe for Mini-Fermenters (OFG-PSt3)



OxyFinger consists of a glass test tube (glass finger) which is coated with an oxygen-sensitive foil. The sensor signal is transmitted to the instrument via a polymer optical fiber.

Cable lengths between 2 and 15 meters are available.

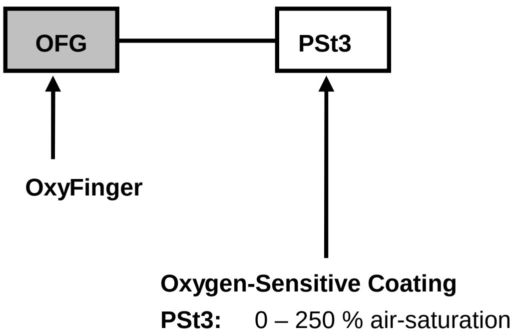
OxyFinger will be manufactured following your specifications. Please specify both length and diameter of the glass finger and the size of the vessel closure.

Please contact our service team directly at ‘info@presens.de’.

The OxyFinger Chemo-Optical DO Probe for Mini-Fermenters has outstanding properties:

- The system can be used after autoclavation without recalibration
- The system is fully autoclavable up to 100 times
- In contrast to classical oxygen electrodes, membrane cleaning and frequent replacement is not necessary
- There are no electrolyte solutions to poison or replenish
- No time for polarization is needed
- Long shelf-life
- measuring range: 0-250 % a.s.
- limit of detection: 0.15 % a.s.

Ordering information



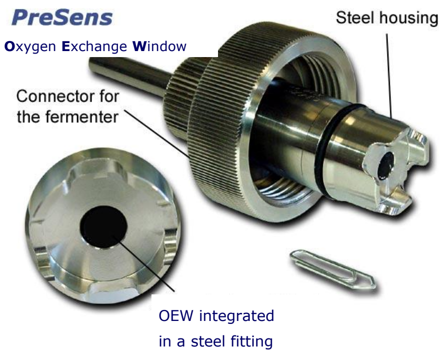
Order code for the OxyFinger: OFG-PSt3

5.2.7 Oxygen Exchange Window (OEW-xx)

Applications:

The **O**xygen **E**xchange **W**indow (OEW) is an oxygen-sensitive coated glass substrate with outstanding properties. It is used to replace the old sensitive coating in the OIM Exchange Cap (OEC) or to integrate in customized steel fittings.

- The PSt3-coated OEW can be used after autoclavation without recalibration
- The PSt3-coated OEW is autoclavable up to 100 times
- In contrast to classical oxygen electrodes, membrane cleaning and frequent replacement is not necessary
- There are no electrolyte solutions to poison or replenish
- No time for polarization needed
- Long shelf-life



The OEW is coated with the oxygen sensitive material PSt3. It has to be integrated into the Oxygen Probe OIM or a customized fitting.

The sensor signal is transmitted to the instrument via a polymer optical fiber. The standard fiber cable length is 2.5 m. If you need a cable length of more than 2.5 m (up to 15 m available) please contact our service team.

Measuring range: 0 – 250 % air-saturation (0 – 22.6 mg/L)

Limit of detection: 0.15 % air-sat. (15 ppb dissolved oxygen)

Ordering information

OEW – PSt3 – DA☐ – DB☐ – H☐ – ☐OP

Oxygen **E**xchange **W**indow

Oxygen-sensitive coating
PSt3:0-50% oxygen

Diameter A
- 13 mm
- 9 mm

Diameter B
- 7 mm
- 6 mm

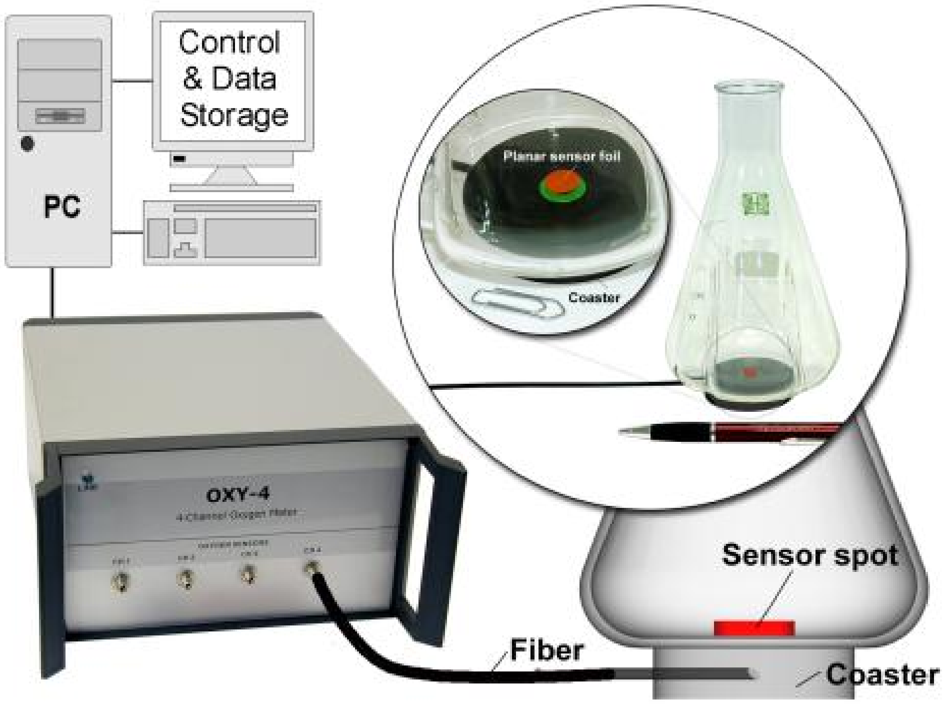
Height
- 6.6 mm
- 6 mm

Optical isolation
- **Y**: with opt. isolation
- **N**: without opt. isolation

Coaster for Shaking Flasks and Spinner Flasks (CSF)

Application:

Online control of oxygen in shaking flasks and spinner flasks.



System set-up for online DO measurement in shaking flasks

Specifications:

The coaster for shaking flasks and Spinner flasks is a tool for online monitoring of dissolved oxygen concentration in shaking flasks. The coaster for shaking flasks does not contain a sensor. It redirects the light so that oxygen sensors in shaking flasks can be read out.

The coaster has a colored circle which makes it easy to position it right under the sensor. The position of the optical read-out is flexible and can be adjusted to different sizes of shaking flasks.

The standard cable length is 2.5 m. The cable has an outer diameter of 2.6 mm.

Please note:

The coaster for shaking flasks and spinner flasks does not contain a sensing layer. It is designed to read out sensor foils which are attached to the inner side of a shaking flask, spinner flask or a similar vessel (e.g. beaker).

Ordering Information:

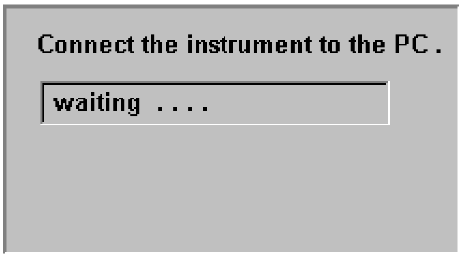
Order code for the Coaster for Shaking Flasks: CS

6 Software

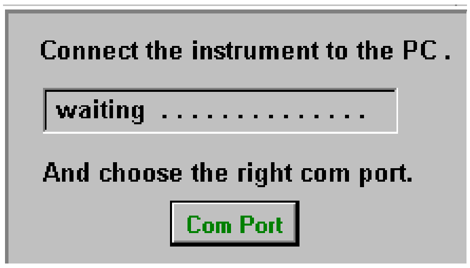
This software is compatible with Windows 95/98/2000/Millenium/NT4.0/XP.

6.1 Software Installation and Starting the Instrument

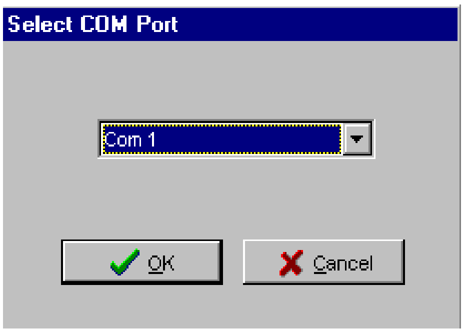
1. Insert the supplied disc/CD into the respective drive. Copy the file PST3v532.exe onto your hard disk (for example, create C:\Fibox3\PST3v532.exe). Additionally, you may create a link (Icon) on your desktop.
2. Connect the Fibox 3 via the supplied serial cable to a serial port of your computer. Tighten the cable with the screws on your computer and on the Fibox 3.
3. Connect the power supply.
4. Please close all other applications as they may interfere with the software. Start the program PS_3V531.exe with a double click. The following information window appears:



5. If the right com port is adjusted this information window disappears within a few seconds. If the wrong com port is adjusted you are asked to set the right com port:



With a left mouse click onto '***Com Port***' you are able to set the right com port. Please confirm your selection by clicking the '***OK***' button. The information window disappears if the right com port is adjusted.

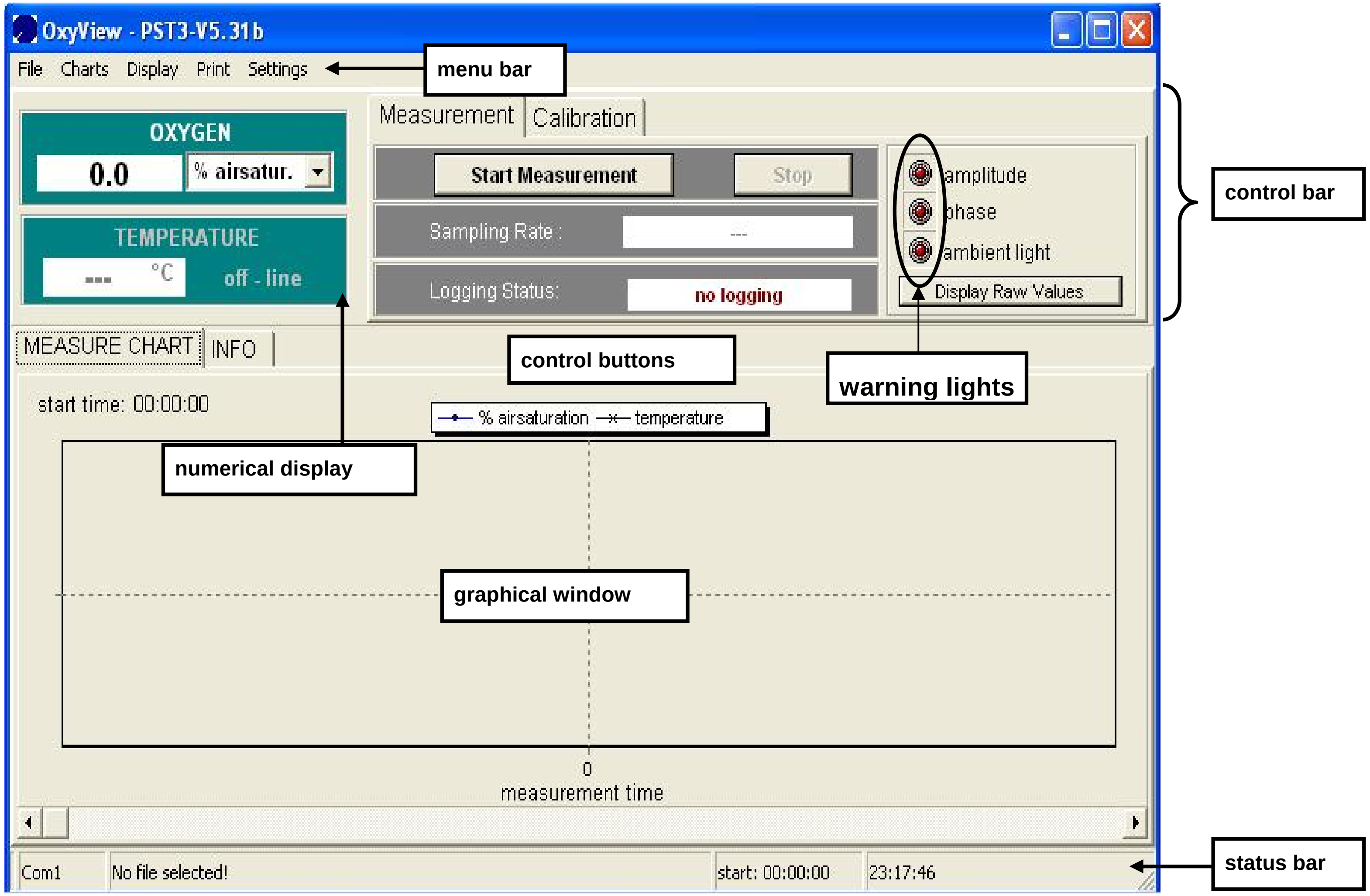


6.2 Function and Description of the Fibox 3 Program

The window shown below is displayed after starting the Fibox 3 software:

The program has 4 main sections:

1. Menu bar
2. Graphical window
3. Status bar
4. Control bar, divided into numerical display, control buttons and warning lights



6.2.1 Menu Bar

The menu bar consists of 5 main parts. Some of them are divided into subwindows.

File	***Charts***	***Display***	***Print***	***Settings***
→ Exit	→ Oxygen	→ Zoom → AutoScaleY1 → Undo Zoom	→ Charts	→ Com Port
	→ Phase			→ Instrument Info
	→ Amplitude	→ Clear Charts		→ analog settings
	→ Temperature	→ Dimensions		→ LED Intensity

File

Exit: Closes the program.

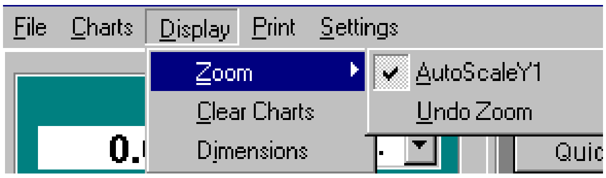
Charts

The respective charts of the measurement can be displayed (√) or hidden

Oxygen: Oxygen content in the chosen unit
Phase: Phase angle, the raw data
Amplitude: The magnitude of the sensor signal
Temperature: The measured temperature

Display

Zoom:



AutoScaleY1 is the default setting. AutoScaleY1 means that the y-axis is scaled automatically.

Undo Zoom: The original display is recovered; see also graphical display

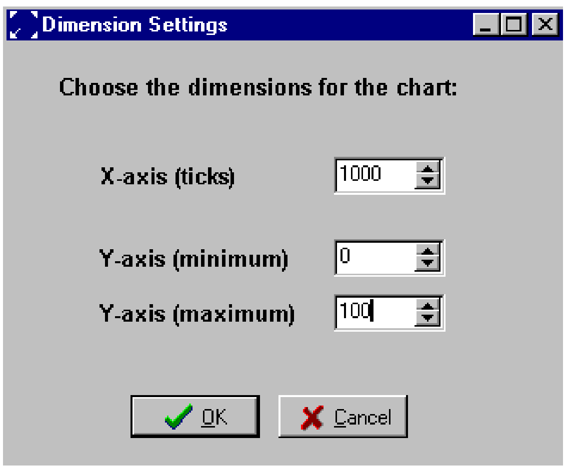
Clear Charts: The graphs shown on the display is cleared.

Dimensions:

You can adjust the number of measurements points on the x-axis shown in the display (maximum number of points are 5000)

Furthermore, you can adjust the minimum and the maximum of the y-axis.

The AutoScaleY1 function is switched off.



Print

Charts: The charts shown in the display can be printed.

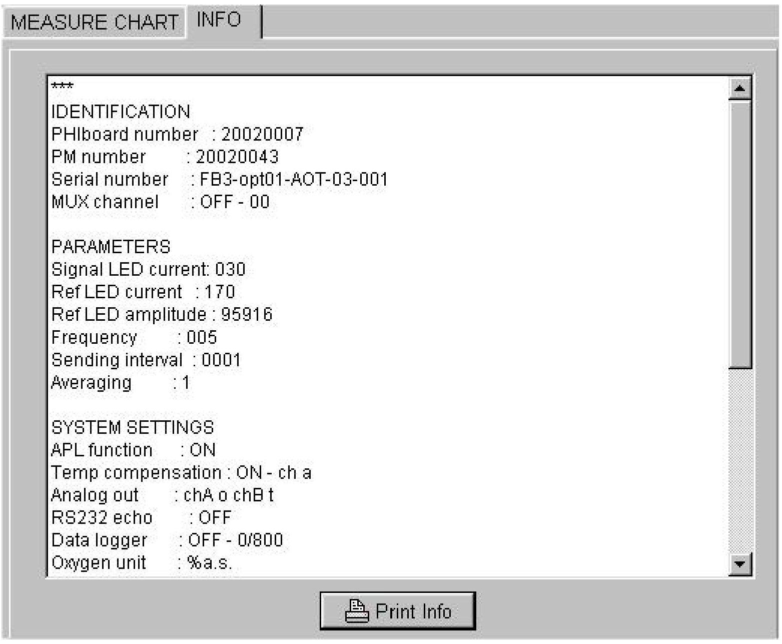
Settings

Com Port: The serial com port (com1 – com20) for the serial interface (RS 232) can be chosen in this window. COM 1 is the default setting. If you choose the wrong com port, the information window *'Connect the instrument to the PC and choose the right com port'* ***does not disappear.***

Instrument Info: Here you can find the version of the software and some important settings of the instrument. If you have a problem with the Fibox 3 oxygen meter, please contact our service team and have the software and instrument information ready.

To change back to the graphical window click the '***Measure Chart***' button.

Instrument Info



Software Info

LED-Intensity

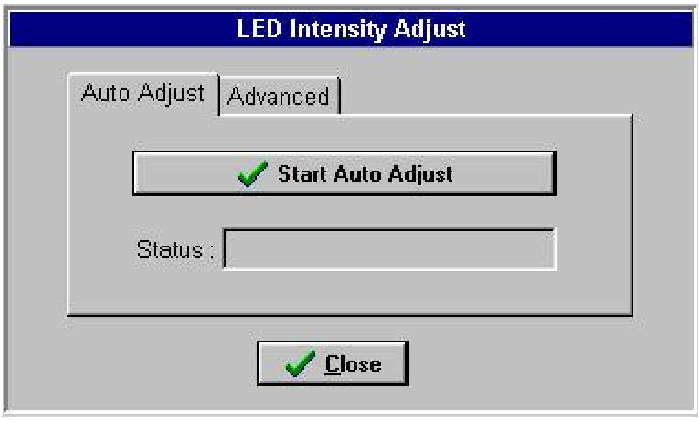
With the current of the LED you can adjust the amount of light illuminating the sensor spot.

You can choose between an '**Auto Adjust**' of the LED where the Fibox 3 adjusts the optimal LED current itself, or you can select '**Advanced**' where you can adjust the LED current yourself.

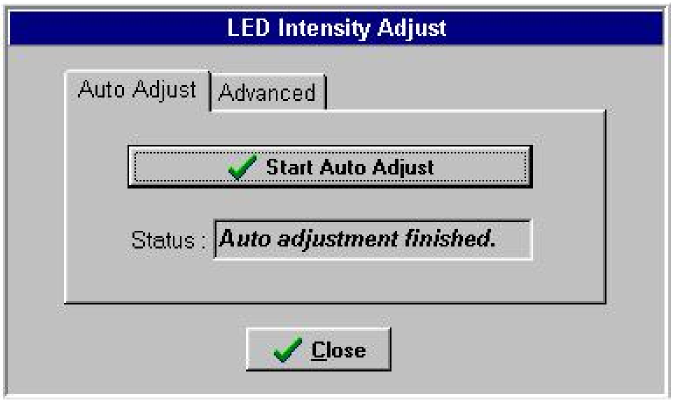
If you increase the LED current, the signal amplitude increases, since a higher light density illuminates the sensor spot.

Auto Adjust:

To make the adjustment of the LED intensity automatically, just click the button '**Start Auto Adjust**'. Please check that the oxygen minisensor has been connected to the instrument.

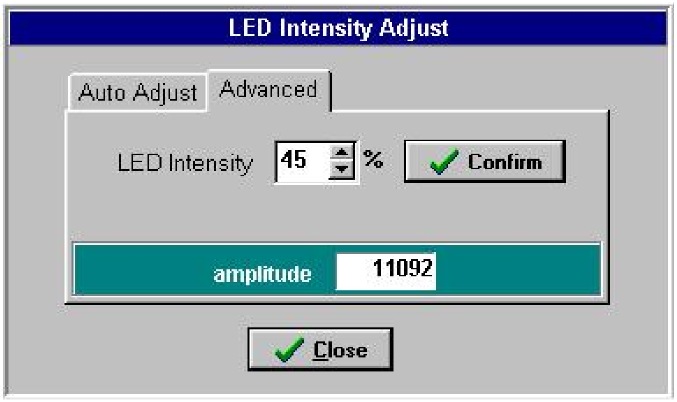


The automatical adjustment of the LED intensity is finished when in the status window the message '**Auto adjustment finished**' appears. Click the '**Close**' button to confirm the settings.

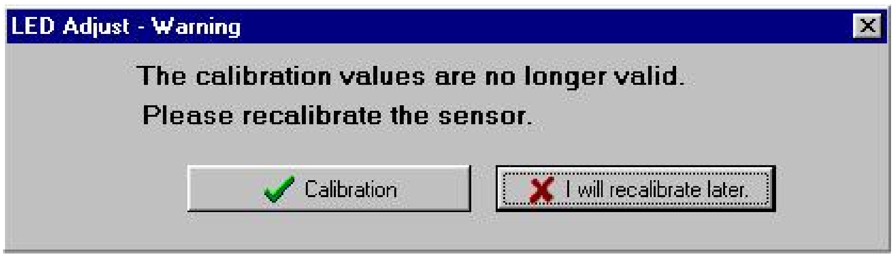


Advanced:

Click the '**Advanced**' button to change the LED current manually. Values between 10 and 100 % are possible. After clicking the 'confirm' button you can see the change of the amplitude in the window below.



Please note: After changing the LED intensity you should re-calibrate the oxygen minisensor. A warning window points you to re-calibrate the oxygen minisensor.



Please note:

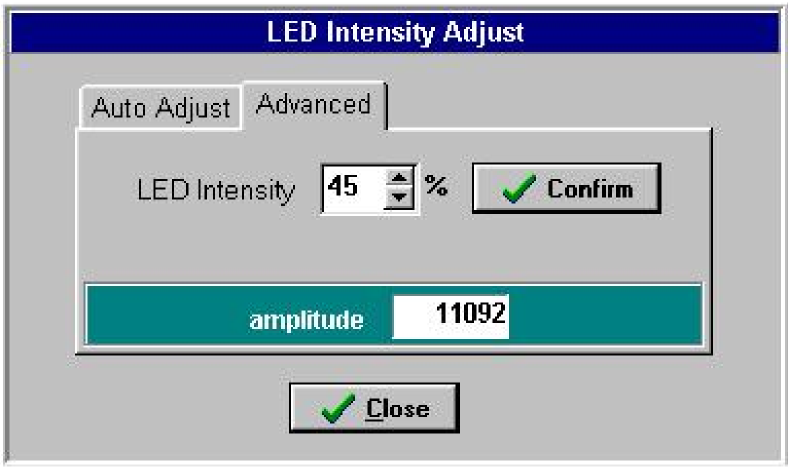
By increasing the light intensity you increase the amplitude of the oxygen minisensor. This leads to smoother phase signals. However, increasing the light intensity can increase photobleaching, which decreases the shelf-life of your sensor.

Analogue output

Here you can choose which data are exported via the analog output. The Fibox 3 device has two analog outputs and one trigger input. The desired data sources (oxygen, temperature, amplitude, phase) can be chosen via the dialog box.

Equivalence coefficient

oxygen	1 : 0.1 (e.g. 973 mV = 97.3 % air saturation)
temperature	1 : 0.1 (e.g. 208 mV = 20.8°C)
amplitude	1 : 20 (e.g. 1110 mV = 22200 relative units)
phase	1 : 0.025 (e.g. 1100 mV = 27.50°)

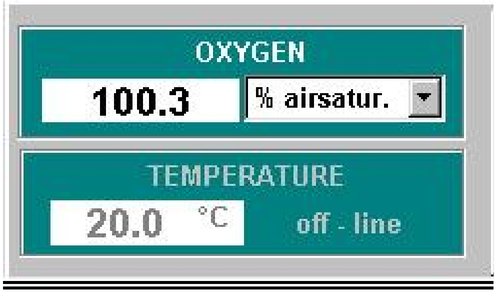


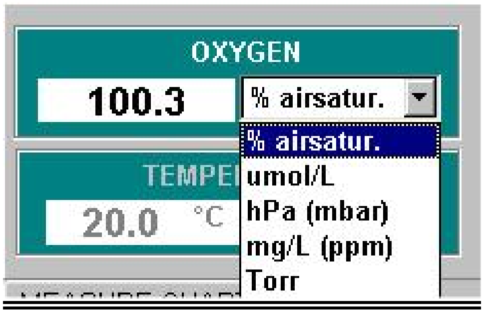
Please note:

If you have adjusted the desired settings of the analog outputs and want to connect the instrument to a datalogger please close the software to store the settings before you disconnect the Fibox 3 from the computer.

6.2.2 Control Bar

Numerical display





The actual oxygen content in the chosen unit (here % air-saturation) is displayed in the oxygen window. The oxygen unit can be changed by clicking the pull down menu. Tables and formulas for the calculation of different concentration scales are given in the appendix.

Please note:

It is also possible to change the oxygen unit during the measurement.

Temperature measurement:

The actual temperature value of the sample (in the case of temperature-compensated measurements) is displayed in the temperature window.

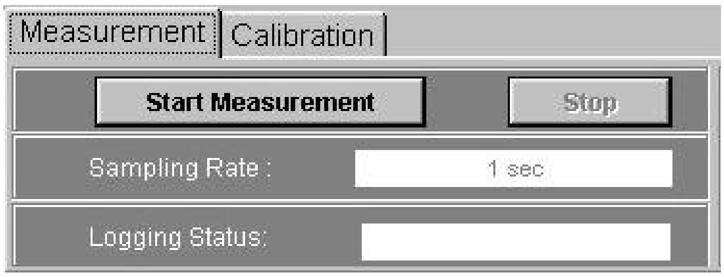
If measurement is performed without temperature compensation, the manually inserted temperature is displayed with the hint that temperature measurement is off–line.

Control buttons:

The way to start a measurement is

(A) Calibration of the minisensor with the Calibration Assistant

(B) Start measurement with the Measurement Assistant



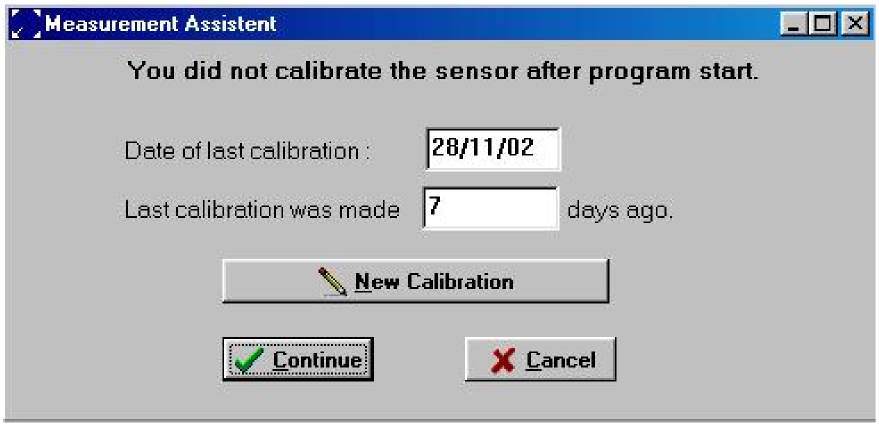
(A) Calibration:

The calibration assistant is opened (see chapter 7, Calibration).

(B) Measurement:

By clicking ‘***Start Measurement***’, the measurement assistant opens to enter the measurement settings.

If you haven't performed sensor calibration yet the following window appears:



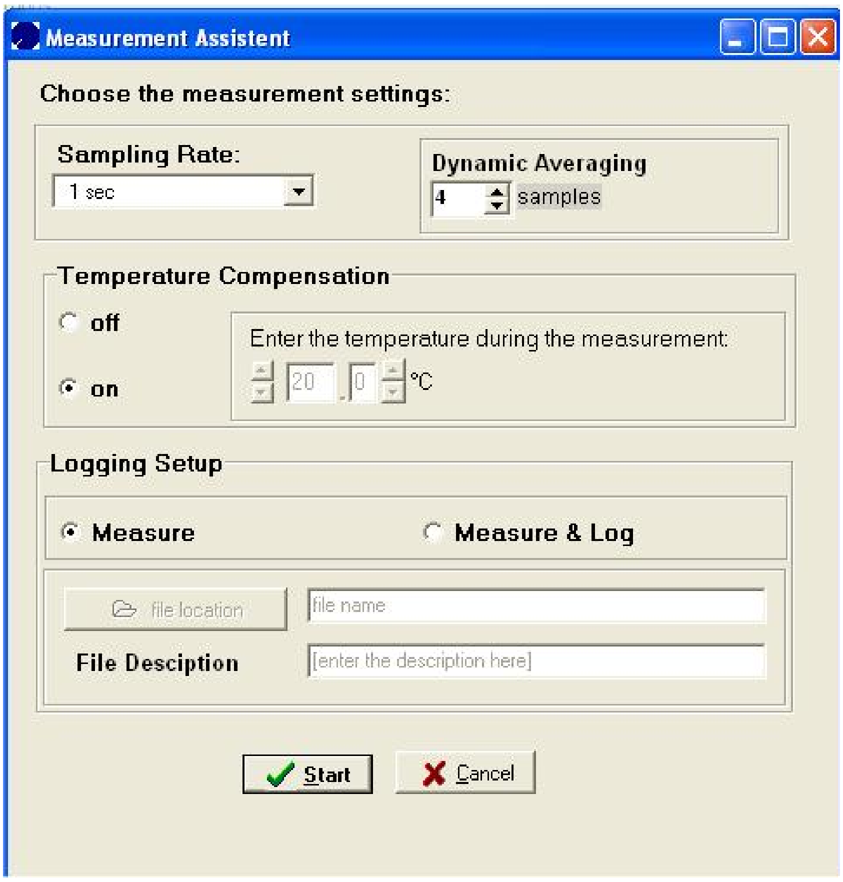
If you want to measure with the last sensor calibration - you can find the '***date of the last calibration***' in the window - click the '***Continue***' button. To obtain reliable results we strongly recommend to perform a sensor calibration before measurement by clicking the '***New calibration***' item when connecting a new sensor.

Follow the instructions given in chapter 7 to calibrate the respective minisensor.

To leave this menu click the '***Cancel***' button.

If you have already performed sensor calibration, the Measurement Assistant will be opened.

In this window you can choose the measurement settings:



In the '**Sampling Rate**' window you can select the desired measurement mode with a drop-down menu.

By clicking the drop-down menu you can choose from '***fast sampling***' (update rate each 250 – 350 ms) to the '***60 min***' mode where each hour a measuring point is recorded.

The speed of recording a measurement point in the '***fast sampling***' mode is about 250 ms when no temperature sensor is connected and decreases to about 350 ms when connecting a temperature sensor or activating the analog output channels.

Please note:

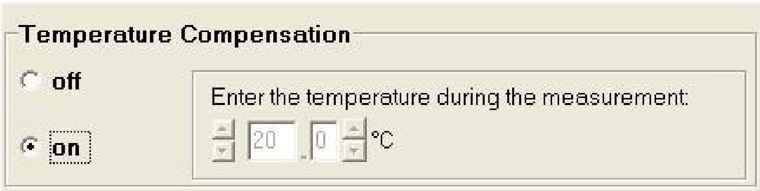
The sensor shelf life can be increased using a slower measuring mode since the effect of photobleaching is reduced. The illumination light is switched off between sampling. A further advantage using a slow measuring mode is that huge amounts of data for long-time measurement can be avoided.

Dynamic averaging

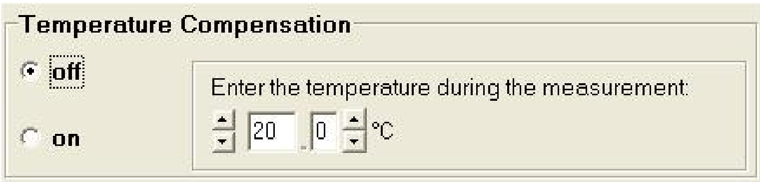
The '***dynamic average***' defines the number of averaged measured values. The higher the running average, the longer the time (sampling time) used for averaging. The higher the running average is set, the smoother the measurement signal (maximum 25 samples). The default setting is 4.

Temperature-compensated oxygen measurements

If you want to measure with temperature compensation, click the '***on***' button. Please ensure that the temperature sensor PT 1000 is connected to the Fibox 3 before you click the '***Start***' button to continue. The window where you can enter the temperature manually is disabled.

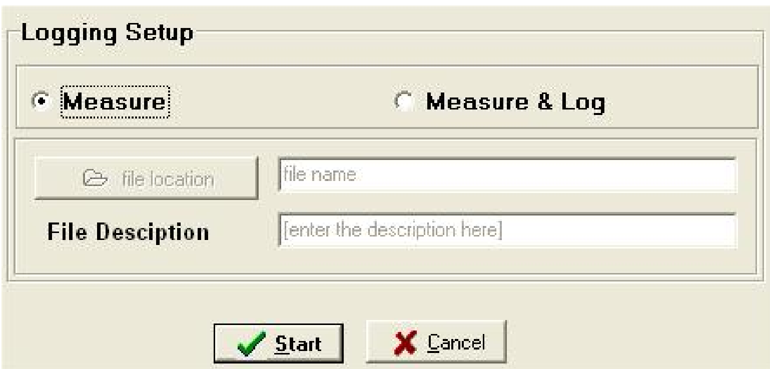


If you want to measure without temperature compensation, choose the '***off***' button. Please enter the temperature of your measurement sample manually. Click the '***Start***' button to start the measurement.

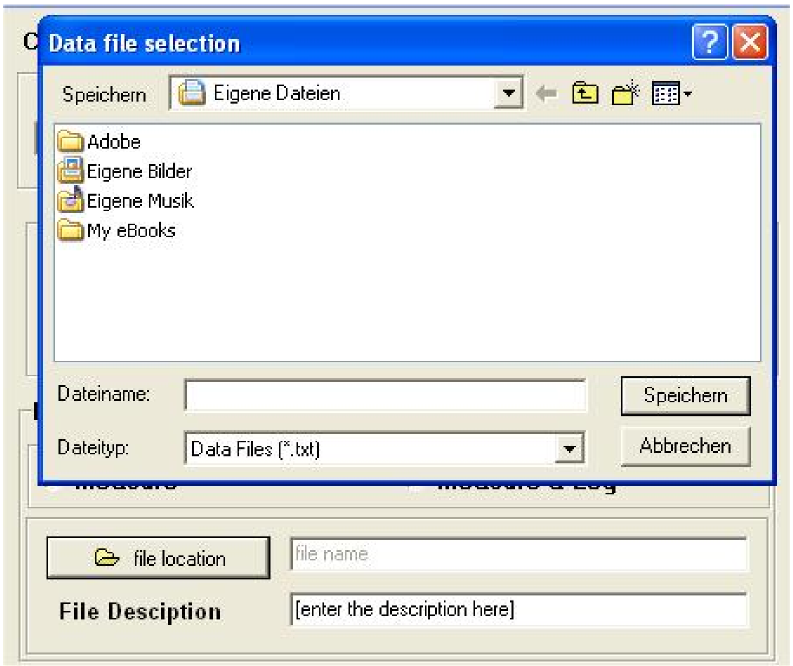


Logging Setup:

To start the measurement without logging data click '***Measure***' in the Logging setup and the '***Start***' button.

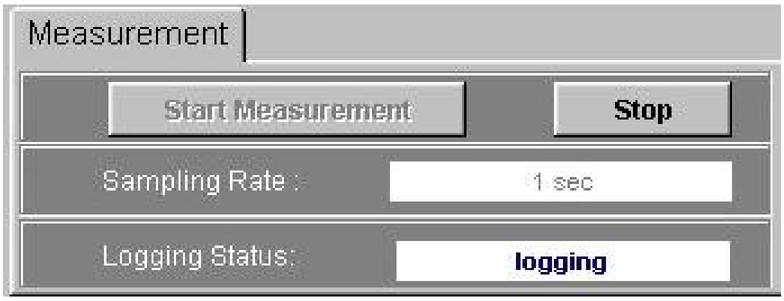


To store the data of your measurement click in the '***Logging Setup***' the '**Measure & Log**' item and click the button '***Choose File***'. Here you can select the location where you want to store the data. Choose as file extension ****.txt***. Click the '***speichern***' ('save') button to confirm your settings.



You can enter a measurement description in the text field '***File description***' which is stored in the ASCII File. To start the measurement, click the '***Start***' button.

In the Information windows '***Sampling Rate***' you can find the adjusted sampling rate. To be sure whether you perform a measurement with or without logging the data, the '***Logging Status***' window displays whether the actual measurement is stored to a file (***logging***) or not (***no logging***).

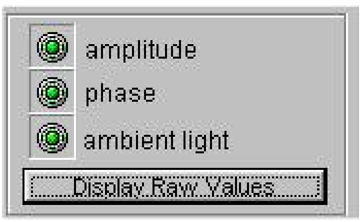


Stop Measurement

The measurement is ended by a left click on the '***stop***' button in the control bar.

Warning Lights:

At the right bottom of the window you can find the amplitude, phase angle and three warning lights. The warning lights are explained below:



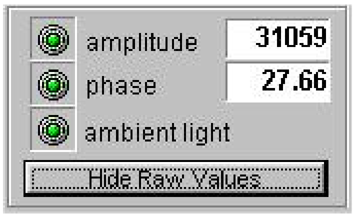
amplitude: red: the amplitude is too low, the sensor tip may be damaged or the sensor cable may not be connected

yellow: the amplitude is critically low, replacement of the sensor is recommended

green: the amplitude is sufficient

phase:	red:	phase angle is out of limits
	green:	phase angle is in normal range
ambient light:	red:	background light (e.g. direct sunlight, lamp) is too high. Decrease of false light is recommended
	green:	ratio of sensor signal to false light is acceptable

By clicking the 'Display Raw Values' button, the raw data of phase angle and amplitude are displayed next to the warning lights.



6.2.3 Graphical Window

The respective sensor signal is displayed according to the selection of the 4 control buttons oxygen, phase, amplitude and temperature (menu chart). The oxygen content is displayed in the chosen unit, the temperature in °C. The raw values (the phase angle in degrees and the sensor amplitude in mV) can also be displayed by clicking the button ***'Display Raw values'***.

Zoom Function:

1. Press the left mouse button and drag from left to right to enlarge a certain area of the graphical window. The graphical window displays the selected data points and is not actualized with new data.
2. Press the left mouse button and drag from right to left to recover the original display, or click the '**Undo Zoom**' button in the *display* menu under *zoom*.

6.2.4 Status Bar

sw1: Displays the serial port which is used for communication of the Fibox 3 device with the PC

sw2: Displays the file name in which the measurement data are stored. „No storage file selected“ is displayed if no file was selected (no data storage).

sw3: Displays the start time of the measurement

sw4: Displays the actual time

6.3 Subsequent Data Handling

In the head of the ASCII file, you find the **description** of your measurement which you have entered when storing the file.

Below you find the '***instrument info***' containing the data of the complete calibration routine and some more important settings of the instrument and firmware.

The '***software info***' below contains the version number of the Fibox 3 software, date and time of the performed measurement. If there is a problem with the Fibox 3 oxygen meter, please contact our service team and have the software and instrument information ready.

Below, you find the '***measure mode settings***' containing the *dynamic averaging*, and the *measuring mode*.

The following rows, separated by semicolons, list the measuring data. The first two rows contain the **date** and **time**, the third the **log-time** in minutes, the fourth the **oxygen content** in the chosen unit. The raw data - **phase angle** in [°] and the **amplitude** in [mV] - are stored in the fifth and sixth row, respectively. The seventh row contains the **temperature** in °C measured by PT1000 temperature sensor. Raw data can be used for user-defined recalculations according to the formulas and tables listed in the appendix.

```
***** DESCRIPTION ***********
Instrument check FB3-AOT-03001

***** INSTRUMENT INFO *******
***
IDENTIFICATION
PHIboard number   : 20020007
PM number         : 20020043
Serial number     : FB3-opt01-AOT-03-001
MUX channel       : OFF - 00

PARAMETERS
Signal LED current: 030
Ref LED current   : 170
Ref LED amplitude : 95916
Frequency         : 005
Sending interval  : 0001
Averaging         : 1

SYSTEM SETTINGS
APL function      : ON
Temp compensation : ON - ch a
Analog out        : chA o chB t
RS232 echo        : OFF
Data logger       : OFF - 0/800
Oxygen unit       : %a.s.

CALIBRATION
Sensor type       : 2
0% a.s. phase 1   : 56.00 at 20.0°C amp 042100
100% a.s. phase 2 : 26.10 at 25.3°C amp 023300
Date  (ddmmyy)    : 300103
Pressure (mBar)   : 1013

FIRMWARE
Code ver 1.081    : 01/23/03, 17:59:49
Xilinx built      : 20/08/02 (MM/DD/YY)
Reset condition   : SLEEP
***
***
***
***** SOFTWARE INFO *********
OxyView - FB3-PST3-V5.02 01/2003
© by PreSens
      11.02.03
      19:47:33

******MEASURE MODE SETTINGS**
Dynamic Aver              1
measure mod   1 sec

start time      19:33:54
date            time/hh:mm:s  logtime/min  oxygen/% air  phase/°   amp     temp/°C
      11.02.03    19:47:33            0        103.43      26.32   14894     22.5
      11.02.03    19:47:33        0.015        104.18      26.24   14914     22.5
      11.02.03    19:47:35        0.032        103.51      26.31   14938     22.5
```

7 Calibration

This chapter describes the calibration of oxygen minisensors containing a **PSt3** oxygen-sensitive coating (measuring range 0 – 250 % air-saturation). To calibrate sensors containing a PSt3 coating you have to use the software PST3v532.exe. For any question, please contact our service team.

7.1 Calibration of Oxygen Dipping Probe (DP-PSt3)

7.1.1 Preparation of the Calibration Standards

Calibration of oxygen minisensors is performed using a conventional two-point calibration in ***oxygen-free water*** (cal 0) and ***water vapor-saturated air*** or ***air-saturated water*** (cal 100).

Preparation of calibration solution 0 (oxygen-free water):

1. Add 1 g sodium sulfite (Na_2SO_3) to the vessel and label it ***cal 0.***
2. Dissolve Na_2SO_3 in 100 mL water.
   The water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is removed by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that the water is oxygen-free.

Keep the vessel closed after calibration with a screw top to minimize oxygen contamination. To prepare oxygen-free water you also can use sodium dithionit ($Na_2S_2O_4$). The shelf life of ***cal 0*** is about 24 hours provided that the vessel has been closed with the screw top.

Preparation of calibration standard 100 (water-vapor saturated air)

1. Place wet cotton wool in a vessel and label it ***cal 100***.
2. Drill two holes for inserting the minisensor and the temperature sensor in the screw top and close the vessel.
3. Wait about 2 minutes to ensure that the air is water vapor-saturated

Alternative preparation of calibration standard 100 (air-saturated water)

1. Add 100 mL water to a suitable vessel and label it ***cal 100***.
2. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (airstone), creating a multitude of small air bubbles, while stirring the solution.
3. After 20 minutes, switch off the air-pump and stir the solution for further 10 minutes to ensure that the water is not supersaturated.

7.1.2 Mounting the Oxygen-Sensitive Minisensors

1. Remove the oxygen sensor carefully from the protective cover.
2. Carefully remove the protective plastic cap covering the oxygen-sensitive sensor spot.
3. Fix the oxygen sensor with a clip to a laboratory support or a similar stable construction.
4. Remove the protective cap from the male fiber plug and connect it to the SMA plug of the Fibox 3 device. The safety nut must be carefully attached while turning slightly clockwise.

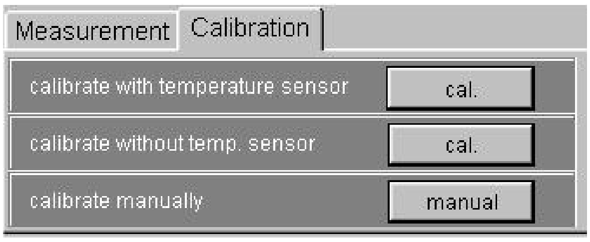
7.1.3 Calibration without Automatic Temperature Compensation

Via the software, you can choose whether to perform the measurement and calibration with or without temperature compensation. If you want to perform the calibration without automatic temperature compensation, please ensure that the delivered temperature sensor PT 1000 is not connected to the Fibox 3.

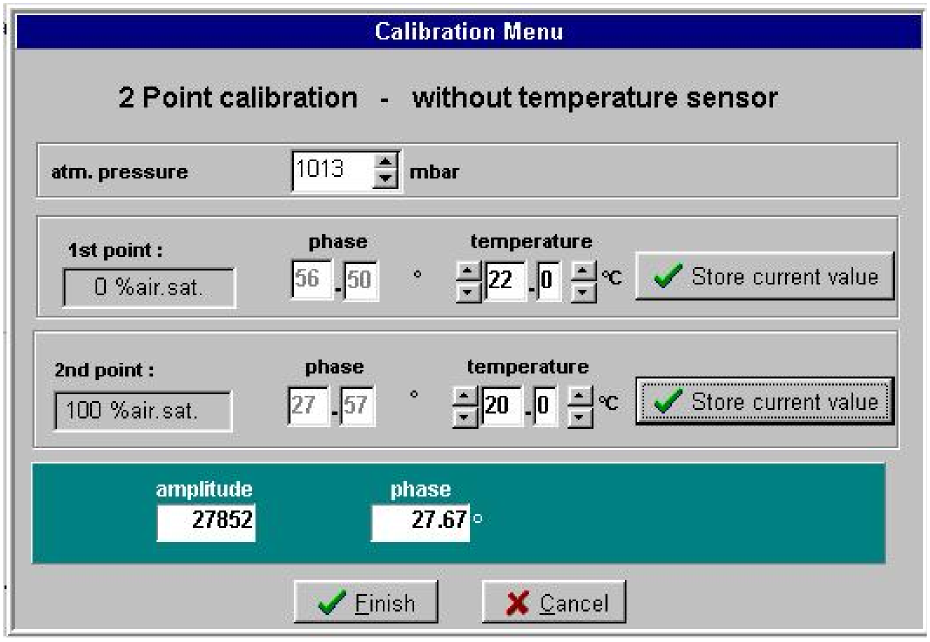
Please note:

Calibration without temperature compensation only makes sense if there is no temperature change during the calibration of the oxygen sensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.

1. Connect the Fibox 3 via the RS232 cable to your computer.
2. Switch on the Fibox 3 and connect the oxygen minisensor as shown in Chapter 7.1.2 "***Mounting the Oxygen-Sensitive Minisensor***“.
3. Start the Fibox 3 software on your computer and click the **calibration** menu item.
4. Select the calibration routine ‘***calibrate without temp sensor***‘ by clicking the ‘***cal.***‘ button



5. Enter the actual **atmospheric pressure** and the **temperature** of the calibration standards. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentration units (mg/L, µmol/L). Please note that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentration units (mg/L, µmol/L), but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.

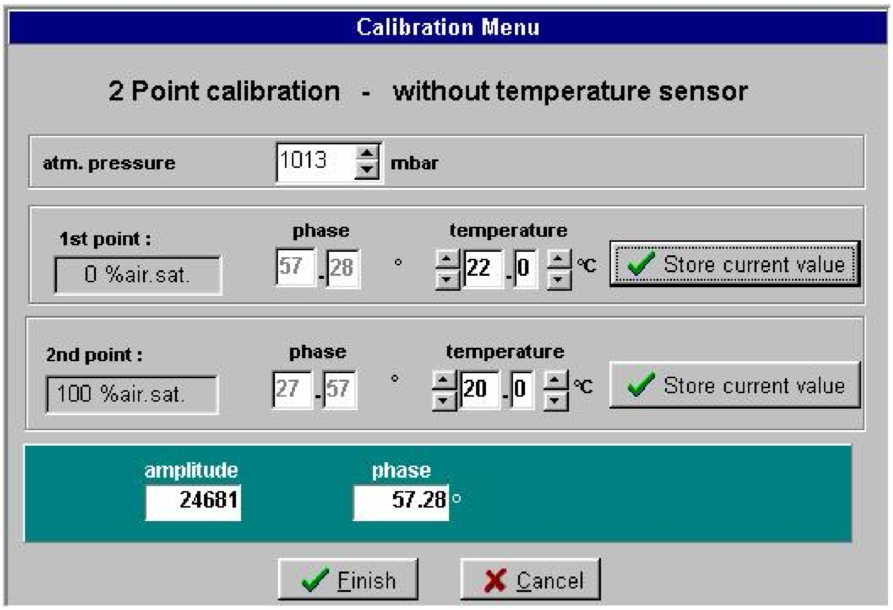


6. Place the calibration standard 100 (***cal 100***), containing wet cotton wool, underneath the oxygen minisensor. The vessel with the label "***cal 100***" has to be closed with the screw top containing the two holes.

   Insert the plastic fiber carefully through one of the holes without touching the oxygen-sensitive spot until it is about 3 cm deep inside the vessel.

   Make sure that the plastic fiber with its sensor spot cannot touch the vessel and the cotton wool.

7. Wait about 3 minutes until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the '***Store current value***' button to store the **100% air-sat.** value at the adjusted temperature.



A message window opens and informs you that you will overwrite the existing calibration values. Click the ‘***Continue***‘ button to store the new calibration data.

8. To record the second calibration value, oxygen-free water (***cal 0***), place the vessel with the label "***cal 0***" underneath the oxygen minisensor.

   Insert the plastic fiber about 2 cm deep into the **cal 0** solution.

   Make sure that the plastic fiber with its sensor spot cannot touch the vessel.

   To increase the response time, stir the ***cal 0*** solution. Wait about 3 minutes until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and click the ‘***Store current value***’ button to store the **0% air-sat.** value at the adjusted temperature.

9. Now calibration is complete. Confirm the calibration values by clicking the ‘***Finish’*** button.

10. Wash the plastic fiber with its sensor spot with distilled water to clean it from sodium sulfite. Exchange the calibration solution 0 with an identical vessel filled with distilled water. Make sure not to touch the sensor spot. Dip the plastic fiber with its sensor spot about 2 cm into a stirred washing solution for about 1 minute. Afterwards, retract the plastic fiber from the vessel without touching the sensor spot.

11. Protect the sensor spot with the delivered protective plastic cover and do not remove it again until just before measurement.

7.1.4 Calibration with Automatic Temperature Compensation

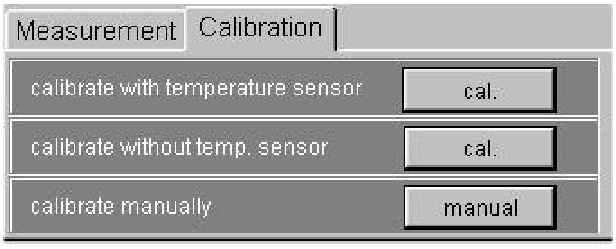
1. Connect the Fibox 3 via the RS232 cable to your computer.

2. To perform temperature-compensated measurement, connect the temperature sensor PT 1000 to the 4-pin connector at the front of the Fibox 3. Fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the minisensor.

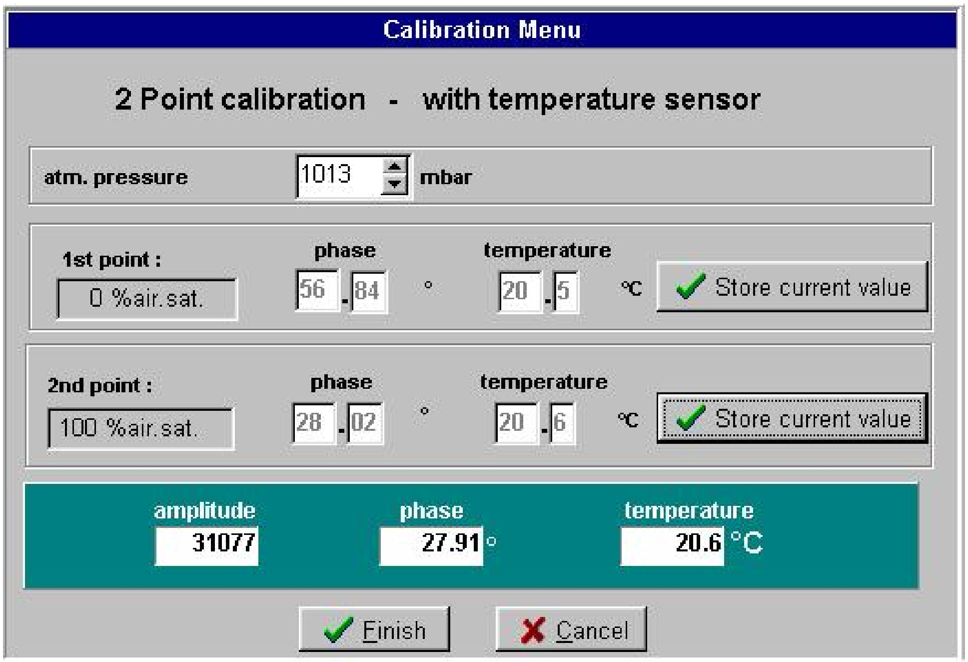
3. Switch on the Fibox 3 and connect the sensor as shown in Chapter 7.1.2 "***Mounting the Oxygen Minisensor***".

4. Start the Fibox 3 software on your computer and click the **calibration** menu item.

5. Select the calibration routine '***calibrate with temperature sensor***' by clicking the respective '***cal.***' button.



6. Enter the '***actual atmospheric pressure***'. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentration units (mg/L, µmol/L). Please note that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentration units (mg/L, µmol/L), but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.



7. Place the calibration standard 100 (***cal 100***), containing wet cotton wool, underneath the oxygen minisensor. The vessel with the label "***cal 100***" has to be closed with the screw top containing the two holes.

   Insert the plastic fiber carefully through one of the holes without touching the oxygen-sensitive spot until it is about 3 cm deep inside the vessel.

   Make sure that the plastic fiber with its sensor spot cannot touch the vessel and the cotton wool.

   Insert the temperature sensor through the second hole. Ensure that there is no temperature gradient between the oxygen dipping probe and the temperature sensor.

8. Wait about 3 minutes until the phase angle and the temperature value are constant (the variation of the phase angle and the temperature should be smaller than ± 0.05° and 0.1 °C, respectively) and press the '***Store current value***' button to store both the '**100*% air-sat.***' and the temperature '***temp at 100%***' values.

   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

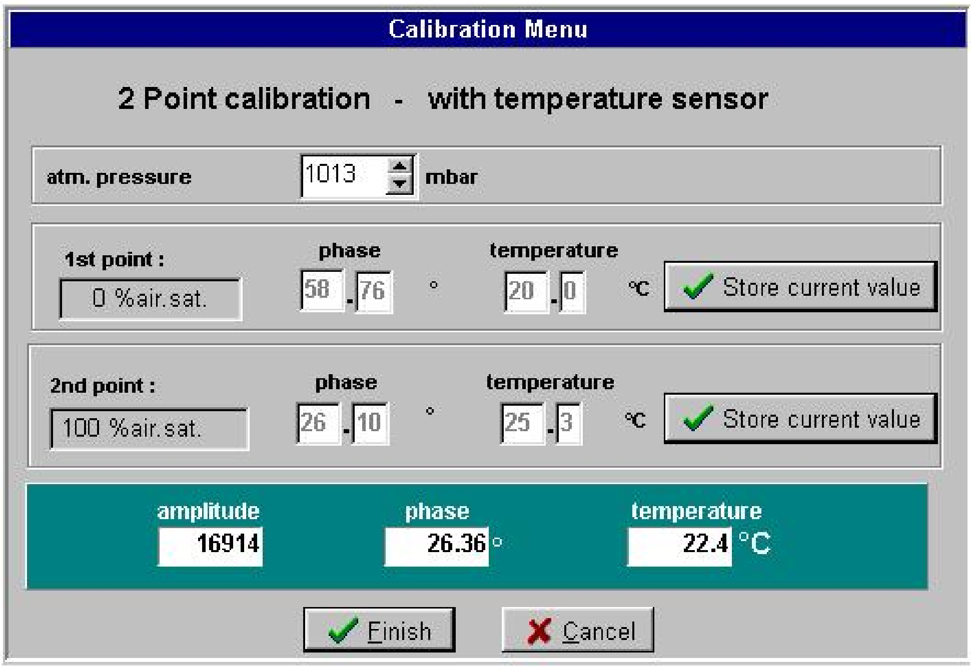
9. To record the second calibration value, oxygen-free water (***cal 0***), place the vessel with the label "***cal 0***" underneath the oxygen minisensor.

   Insert the plastic fiber about 2 cm deep into the ***cal 0*** solution.

   Insert the temperature sensor into the ***cal 0*** calibration vessel.

   Make sure that the plastic fiber with its sensor spot cannot touch the vessel.

   To increase the response time, stir the ***cal 0*** solution. Wait about 3 minutes until the phase angle and the temperature are constant (the variation of the phase angle and the temperature should be smaller than ± 0.05° and 0.1 °C, respectively) and click the '***Store current value***' button to store the '**0% air-sat.**' and **temp. at 0%** values.



A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

10. Now, calibration with temperature compensation is complete. Confirm the calibration values by clicking the '***Finish***' button.

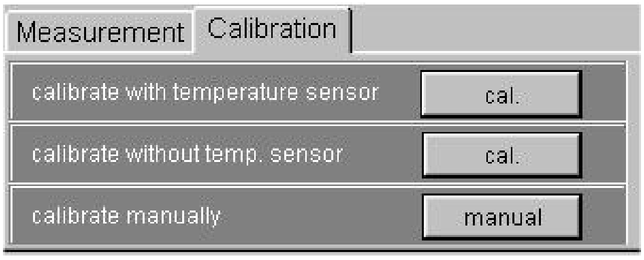
11. Wash the plastic fiber with its sensor spot and the temperature sensor with distilled water to clean it from sodium sulfite. Exchange the calibration solution 0 with an identical vessel filled with distilled water. Make sure not to touch the sensor spot. Dip the plastic fiber with its sensor spot and the temperature sensor about 2 cm into a stirred washing solution for about 1 minute. Afterwards, retract the plastic fiber from the vessel without touching the sensor spot.

12. Protect the sensor spot with the delivered protective plastic cover and do not remove it again until just before measurement.

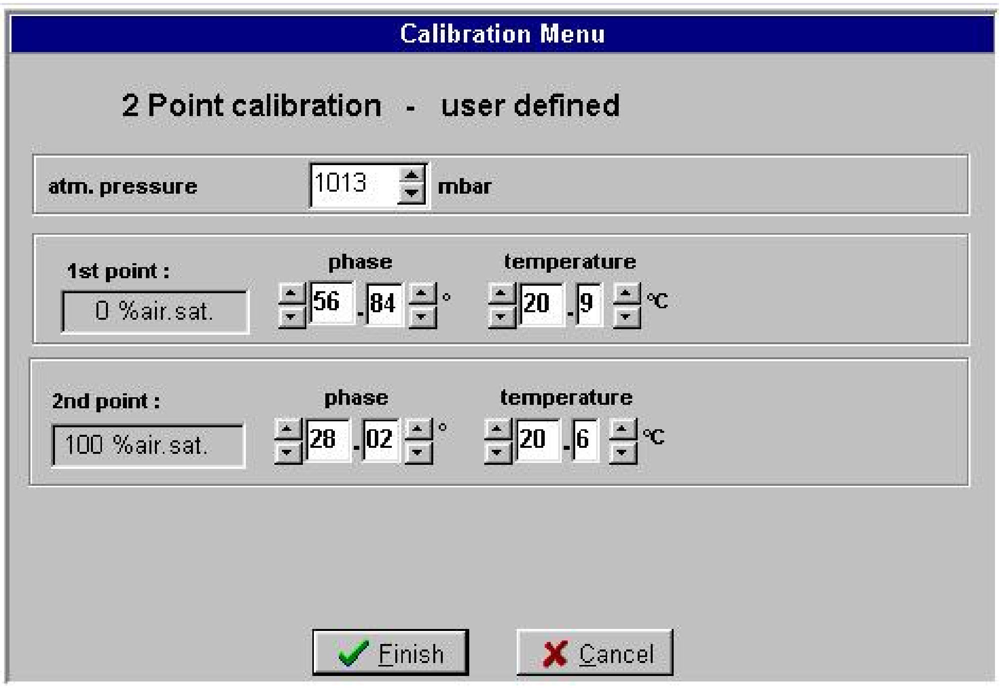
7.1.5 Manual Calibration

A manual calibration should be applied if you do not want to calibrate your sensor again. However, this is only possible if you already know the calibration values of the special sensor.

1. Connect the Fibox 3 via the RS232 cable to your computer.
2. Switch on the Fibox 3 oxygen meter.
3. Start the Fibox 3 software on your computer and click the **Calibration** menu item.
4. Select the calibration routine '**calibrate manually**' by clicking the **manual** button.



5. Enter the **atmospheric pressure** at which calibration was performed (**not** the **actual** one) and the respective calibration values **0% air-sat., temp. at 0%** and **100 % air-sat., temp. at 100%**.



6. Now the user-defined calibration is complete. Confirm the calibration values by clicking the **Finish** button. A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

7.2 Calibration of Flow-Through Cell FTC-PSt3

7.2.1 Preparation of the Calibration Standards

Calibration of the minisensors is performed using a conventional two-point calibration in ***oxygen-free water*** (cal 0) and ***air-saturated water*** (cal 100).

Preparation of calibration solution 0 (oxygen-free water):

1. Add 1 g sodium sulfite (Na_2SO_3) to a vessel and label it ***cal 0***.
2. Dissolve Na_2SO_3 in 100 mL water.

   The water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is removed by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that the water is oxygen-free.

Keep the vessel closed with a screw top after calibration to minimize oxygen contamination

To prepare oxygen-free water you also can use sodium dithionit ($Na_2S_2O_4$). The shelf life of ***cal 0*** is about 24 hours provided that the vessel has been closed with the screw top.

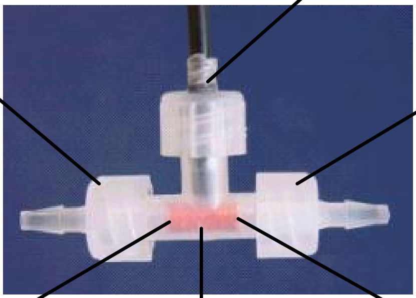
Preparation of calibration solution 100 (air-saturated water)

1. Add 100 mL water to a suitable vessel and label it ***cal 100***.
2. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (airstone), creating a multitude of small air bubbles, while stirring the solution.
3. After 20 minutes, switch off the air-pump and stir the solution for further 10 minutes to ensure that the water is not supersaturated.

7.2.2 Mounting the Flow-Through Cell Oxygen Sensors

1. Remove the flow-through cell oxygen sensor carefully from the protective cover. The oxygen-sensitive material is immobilized to a glass tube which is located in a T-connector with two female Luer-Lock adapters. The glass tube is tightened with a viton seal and two male Luer-Lock connectors fix the glass tube in the T-connector. On request the seal can also be made from silicone.

   Do not remove the two male Luer-Lock adapters from the T-connector. You may loose the viton seal and the glass tube may be dislocated.



2. Fix the male Luer-Lock adapter with the integrated 2 mm POF (polymer optical fiber) to the Luer T-connector and ensure that the fiber is located close to the glass tube.

3. Fix the flow-through cell with a clip to a laboratory support or a similar stable construction.

4. Connect the two male Luer-Lock connectors with the tubings of your flow-through system.

5. Remove the protective cap from the male fiber plug and connect it to the SMA plug of the Fibox 3 device. The safety nut must be carefully attached while turning slightly clockwise.

7.2.3 Calibration without Automatic Temperature Compensation

Via the software you can choose whether to perform the measurement and calibration with or without temperature compensation. If you want to perform the calibration without automatic temperature compensation, please ensure that the delivered temperature sensor PT 1000 is not connected to the Fibox 3 oxygen meter.

Please note:

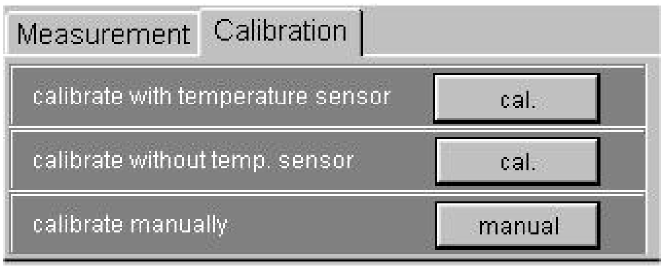
Calibration without temperature compensation only makes sense if there is no temperature change during the calibration of the oxygen sensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.

1. Connect the Fibox 3 via the RS232 cable to your computer.

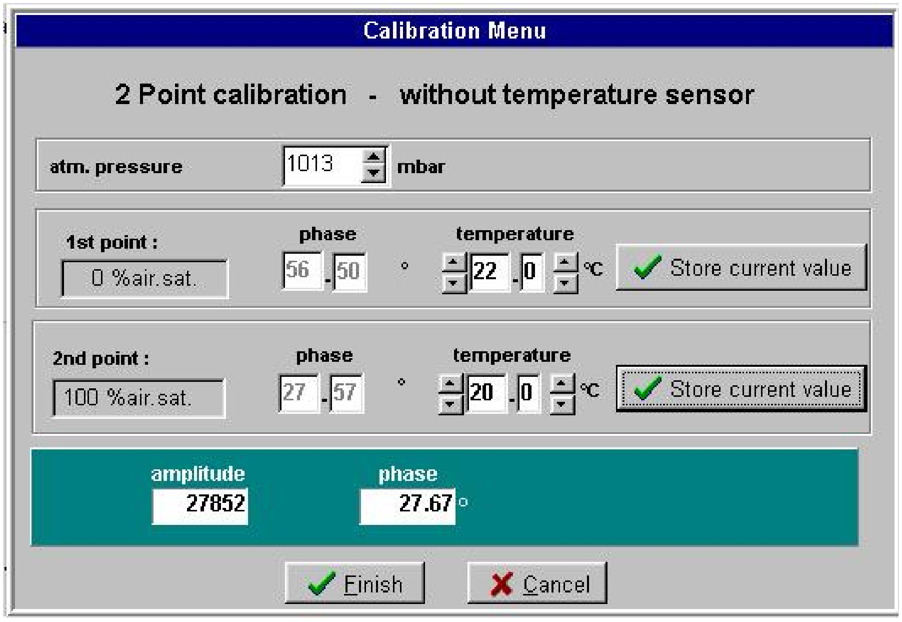
2. Switch on the Fibox 3 and connect the sensor as shown in Chapter 7.2.2 "***Mounting the Flow-Through Cell Oxygen Sensor***“.

3. Start the Fibox 3 software on your computer and click the **calibration** menu item.

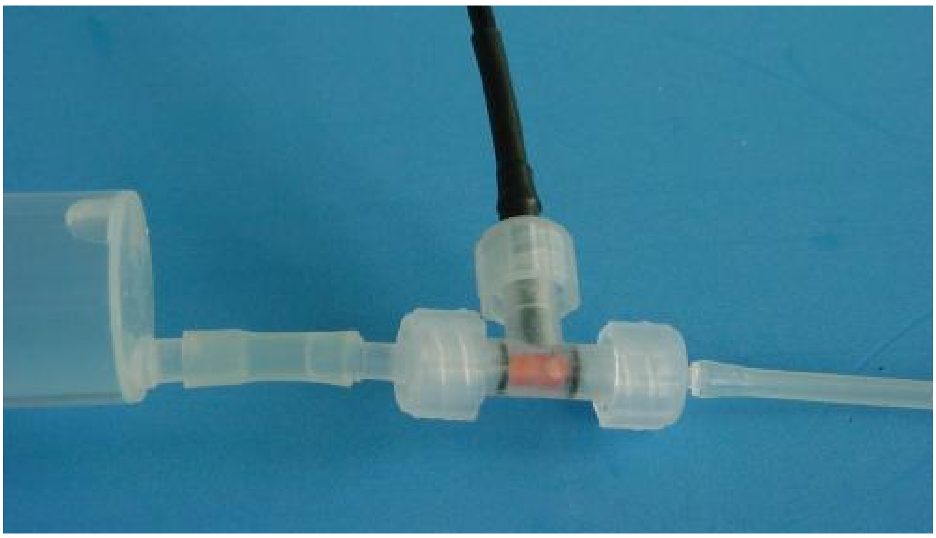
4. Select the calibration routine ‘**calibrate without temp. sensor**’ by clicking the respective ’***cal.***’ button.



5. Enter the ’***actual atmospheric pressure***’ and the ’***temperature***’ of the calibration standards. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentration units (mg/L, µmol/L). Please note that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentration units (mg/L, µmol/L), but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.



6. Connect one plastic tubing with a syringe, the other dip into the vessel containing the calibration solution 100, ***cal 100***. Fill the syringe slowly with calibration solution 100. Please ensure that there are no air-bubbles located in the glass tube of the flow-through cell.



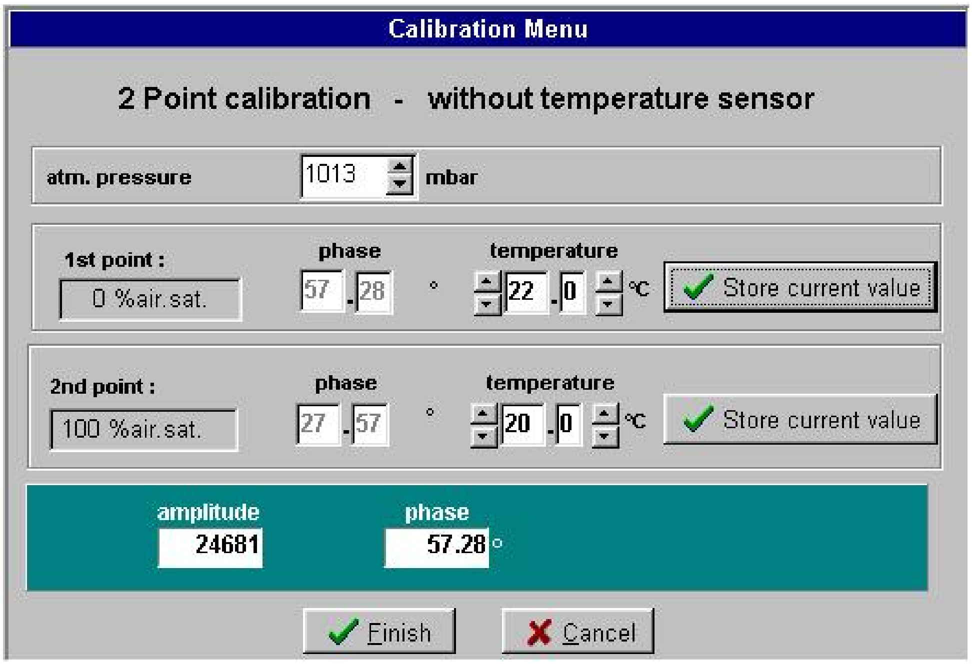
7. Wait about 3 minutes until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the '***Store current value***' button to store the **100% air-sat.** value at the adjusted temperature. Afterwards, press the calibration solution into the waste.

   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

8. To record the second calibration value, oxygen-free water, dip the plastic tubing into the vessel containing the calibration solution 0, ***cal 0*** and fill the syringe slowly with it. Please ensure that there are no air-bubbles located in the glass tube of the flow-through cell.

   Wait about 3 minutes until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and click the '**Store *current value***' button to store the **0% air-sat.** value at the adjusted temperature. Afterward, press the calibration solution into the waste.

   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.



9. Now calibration is complete. Confirm the calibration values by clicking the '***Finish'*** button.

10. Wash the flow-through cell with distilled water to clean it from sodium sulfite. Dip the plastic tubing into a vessel containing distilled water and fill the syringe. Press the washing solution to the waste. Repeat this washing procedure 3 times.

7.2.4 Calibration with Automatic Temperature Compensation

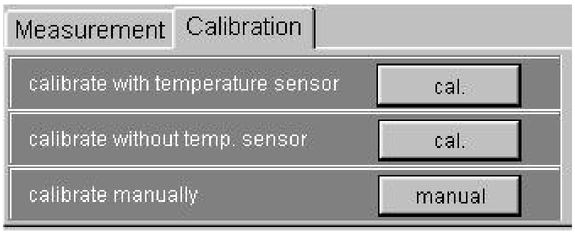
1. Connect the Fibox 3 via the RS232 cable to your computer.

2. To perform temperature-compensated measurement, connect the temperature sensor PT 1000 to the 4-pin connector at the front of the Fibox 3.

3. Switch on the Fibox 3 and connect the sensor as shown in Chapter 7.2.2 "***Mounting the Flow-Through Cell Oxygen Sensor***“.

4. Start the Fibox 3 software on your computer and click the calibration menu item.

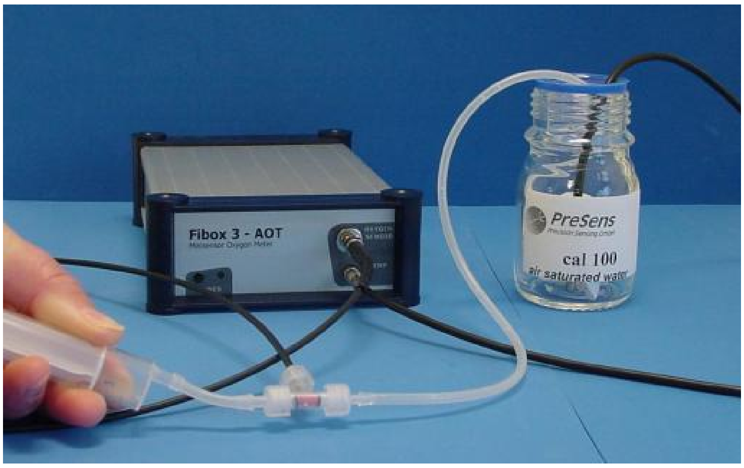
5. Select the calibration routine ‘***calibrate with temperature sensor***‘ by clicking the respective ‘***cal.***‘ button.

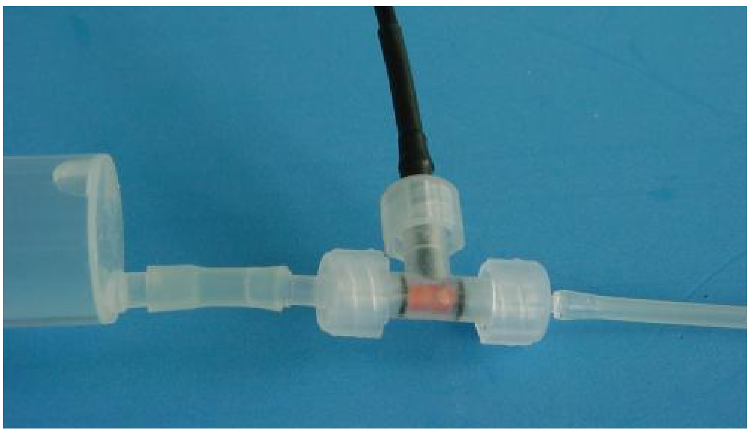


6. Enter the ‘***actual atmospheric pressure***’. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentration units (mg/L, µmol/L). Please note that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentration units (mg/L, µmol/L), but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.

7. Connect one plastic tubing with a syringe, the other dip into the vessel containing the calibration solution 100, "***cal 100***". Fill the syringe slowly with calibration solution 100. Please ensure that there are no air-bubbles located in the glass tube of the flow-through cell.

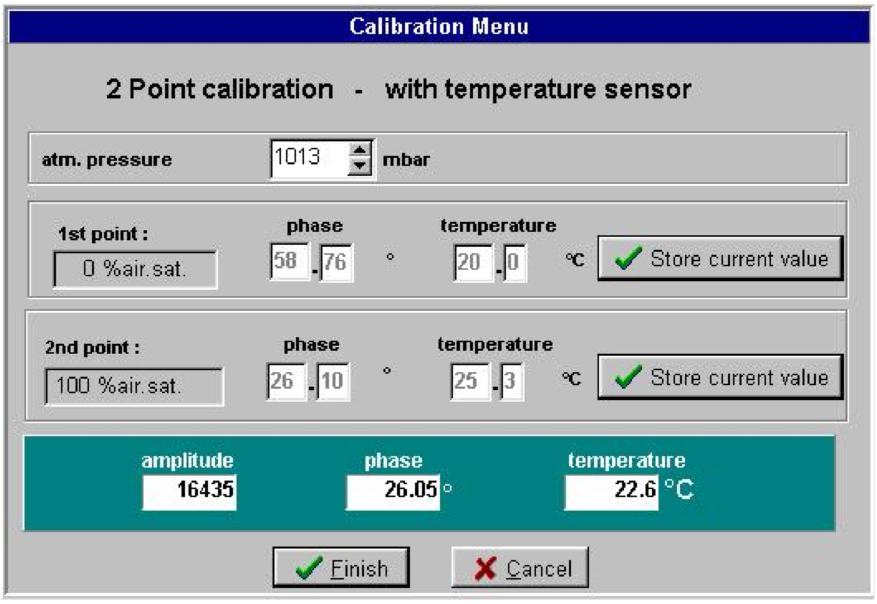
   Insert the temperature sensor into the calibration solution "***cal 100***". Ensure that there is no temperature gradient between the flow-through cell and the temperature sensor.





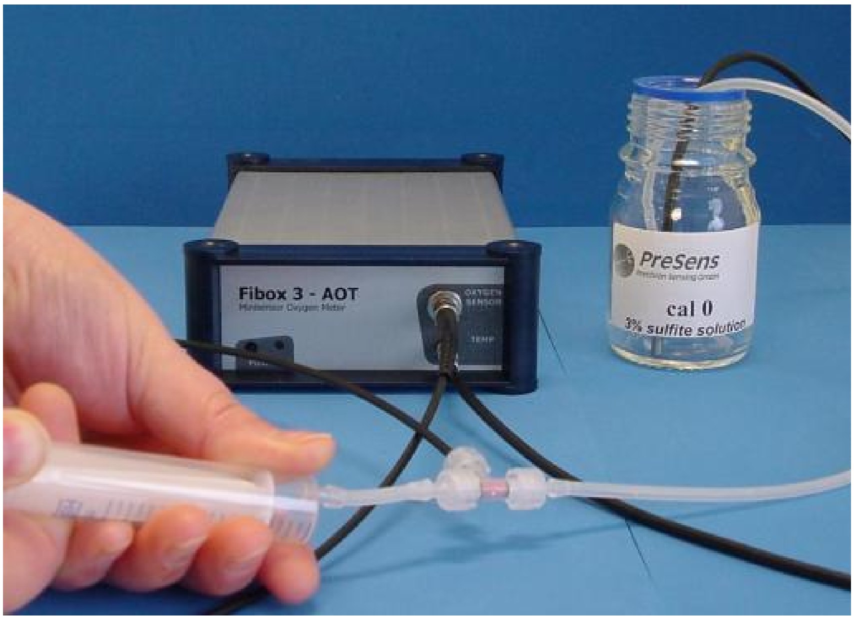
7. Wait about 3 minutes until the phase angle and the temperature value are constant (the variation of the phase angle and the temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and press the '***Store current value***' button to store both the ***100% air-sat.*** and its temperature '***temp at 100%***'. Afterwards, press the calibration solution into the waste.

A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

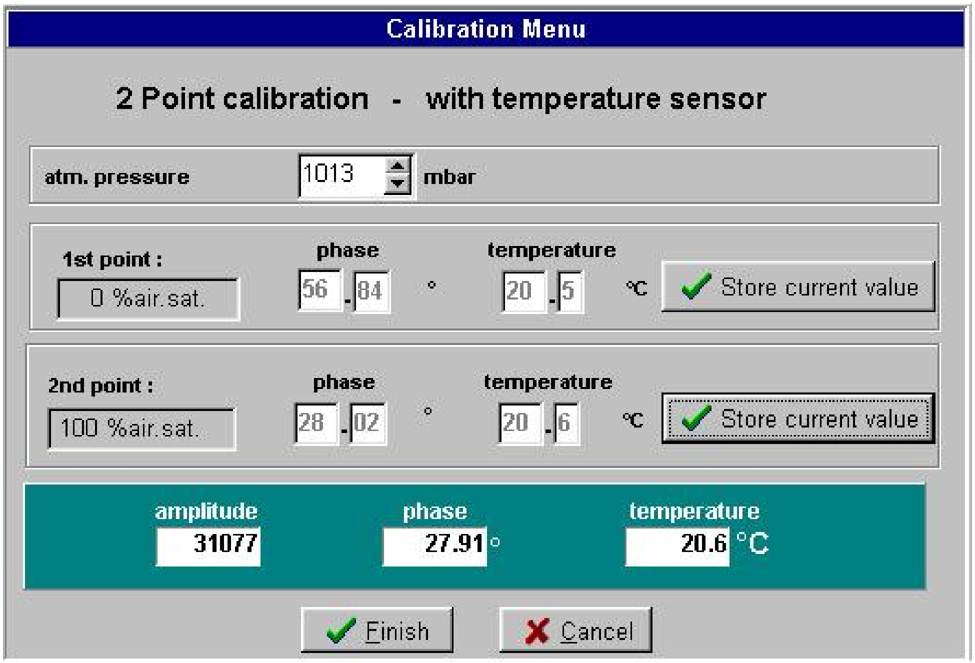


9. To record the second calibration value, oxygen-free water, dip the plastic tubing into the vessel containing the calibration solution 0, ***cal 0,*** and fill the syringe slowly with it. Please ensure that there are no air-bubbles located in the glass tube of the flow-through cell.

Insert the temperature sensor into the calibration solution ***cal 0***. Ensure that there is no temperature gradient between the flow-through cell and the temperature sensor.



9. Wait about 3 minutes until the phase angle and the temperature are constant (the variation of the phase angle and temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and click the '***Store current value***' button to store the **0% air-sat.** and **temp. at 0%** values. Afterwards, press the calibration solution into the waste.



Again, a message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

11. Now calibration with temperature compensation is complete. Confirm the calibration values by clicking the '***Finish'*** button.

12. Wash the flow-through cell with distilled water to clean it from sodium sulfite. Dip the plastic tubing into a vessel containing distilled water and fill the syringe. Press the washing solution to the waste, not back into the vessel. Please repeat this washing procedure 3 times.

    Also wash the temperature sensor by dipping it into water.

7.2.5 Manual Calibration

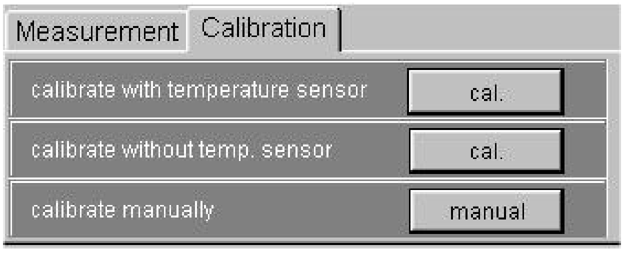
A manual calibration should be applied, if you do not want to calibrate your sensor again. However, this is only possible if you already know the calibration values of the special sensor.

1. Connect the Fibox 3 via the RS232 cable to your computer.

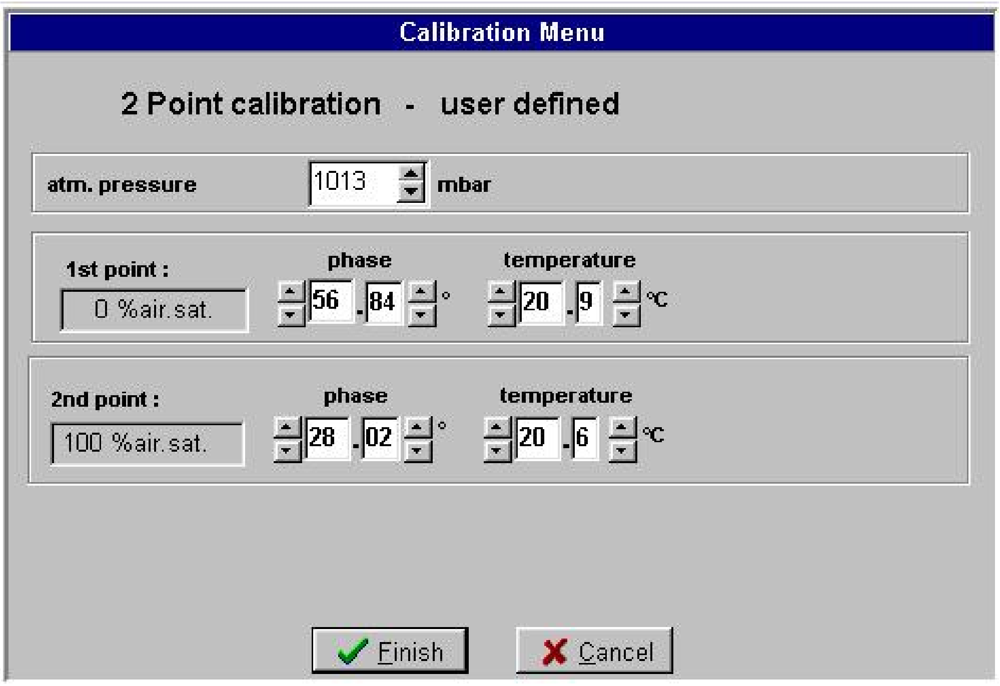
2. Switch on the Fibox 3 oxygen meter.

3. Start the Fibox 3 software on your computer and click the **Calibration** menu item.

4. Select the calibration routine '**calibrate manually**' by clicking the **manual** button.



5. Enter the **atmospheric pressure** at which calibration was performed (**not** the **actual** one) and the respective calibration values **0 % air-sat., temp. at 0 %** and **100 % air-sat., temp. at 100%**.



6. Now the user-defined calibration is complete. Confirm the calibration values by clicking the **Finish** button. A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

7.3 Calibration of Planar Oxygen-Sensitive Foils (SP-PSt3)

7.3.1 Preparation of the Calibration Standards

Calibration of the minisensors is performed using conventional two-point calibration with ***oxygen-free water*** (cal 0) and ***water vapor-saturated air*** or ***air-saturated water*** (cal 100).

Preparation of calibration solution 0 (oxygen-free water):

1. Add 1 g sodium sulfite(Na_2SO_3) to a vessel and label it **cal 0.**
2. Dissolve Na_2SO_3 in 100 mL water.

   The water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is eliminated (removed) by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that the water is oxygen-free.

Keep the vessel closed with the screw top after calibration to minimize oxygen contamination

To prepare oxygen-free water you also can use sodium dithionit ($Na_2S_2O_4$). The shelf life of **cal 0** is about 24 h provided that the vessel has been closed with the screw top.

Preparation of calibration standard 100 (water vapor-saturated air)

1. Place wet cotton wool in a vessel and label it **cal 100**.
2. Drill two holes for inserting the minisensor and the temperature sensor in the screw top and close the vessel with it.
3. Wait about 2 minutes to ensure that the air is water vapor-saturated

Alternative preparation of calibration solution 100 (air-saturated water)

1. Add 100 mL water to a suitable vessel and label it ***cal 100***.
2. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (airstone), creating a multitude of small air bubbles, while stirring the solution.
3. After 20 minutes, switch off the air-pump and stir the solution for further 10 minutes to ensure that the water is not supersaturated.

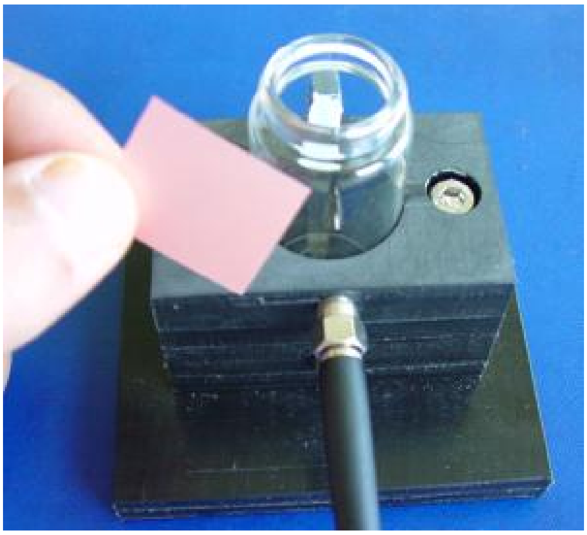
7.3.2 Mounting Planar Oxygen-Sensitive Foils

1. Remove the oxygen-sensitive foil carefully from the protective cover.

2. Glue small spots of the oxygen-sensitive foil into the desired glass vessel.

Please note: Be sure to glue the sensor spots onto your vessel with the proper side! The sensor support (polyester foil or glass) on which the sensor is spotted (identifiable by its faint reflection) is glued to the vessel, while the sensor itself must look toward the sample. The figure on page 12 shows how the (highly enlarged) sensor spot must be glued to the vessel.

3. Remove the protective cap from the male fiber plugs of the delivered fiber cable and connect it to the SMA plugs of the Fibox 3 device and the holding device. The safety nut must be carefully attached while turning slightly clockwise.



7.3.3 Calibration without Automatic Temperature Compensation

Via the software, you can choose whether to perform the measurement and calibration with or without temperature compensation. If you want to perform the calibration without automatic temperature compensation, please ensure that the delivered temperature sensor PT 1000 is not connected to the Fibox 3.

Please note:

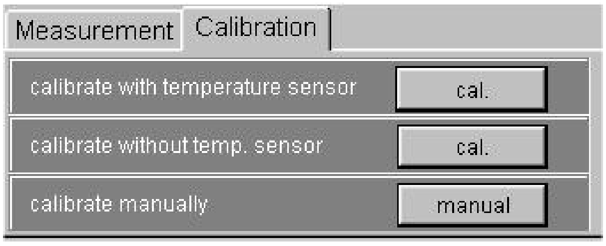
Calibration without temperature compensation only makes sense if there is no temperature change during the calibration of the oxygen minisensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.

1. Connect the Fibox 3 via the RS232 cable to your computer.

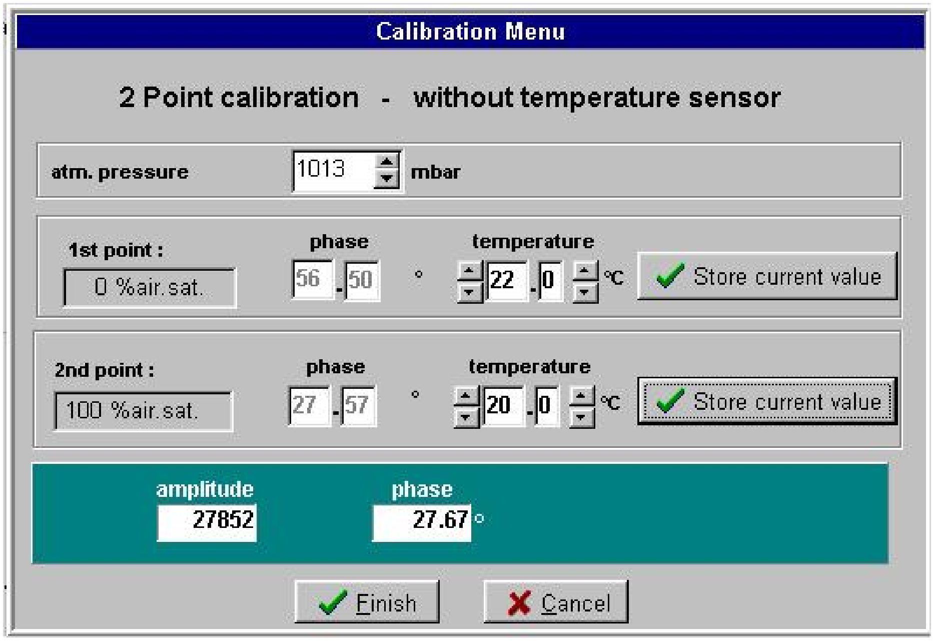
2. Switch on the Fibox 3 and connect the oxygen minisensor as shown in Chapter 7.3.2 "***Mounting Planar Oxygen-Sensitive Foils***“.

3. Start the Fibox 3 software on your computer and click the **calibration** menu item.

4. Select the calibration routine ‘***calibrate without temp. sensor***‘ by clicking the respective ‘***cal.***‘ button.



5. Enter the actual ‘***atmospheric pressure***’ and the ‘***temperature***’ of the calibration standards. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentration units (mg/L, µmol/L). Please note that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentration units (mg/L, µmol/L), but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.



6. Place the calibration solution 100 (**cal 100**), air-saturated water (or water vapor-saturated air), into the glass vessel. To minimize the response time, slightly stir the solution. Please ensure that the **cal 100** solution completely covers the sensor foil.

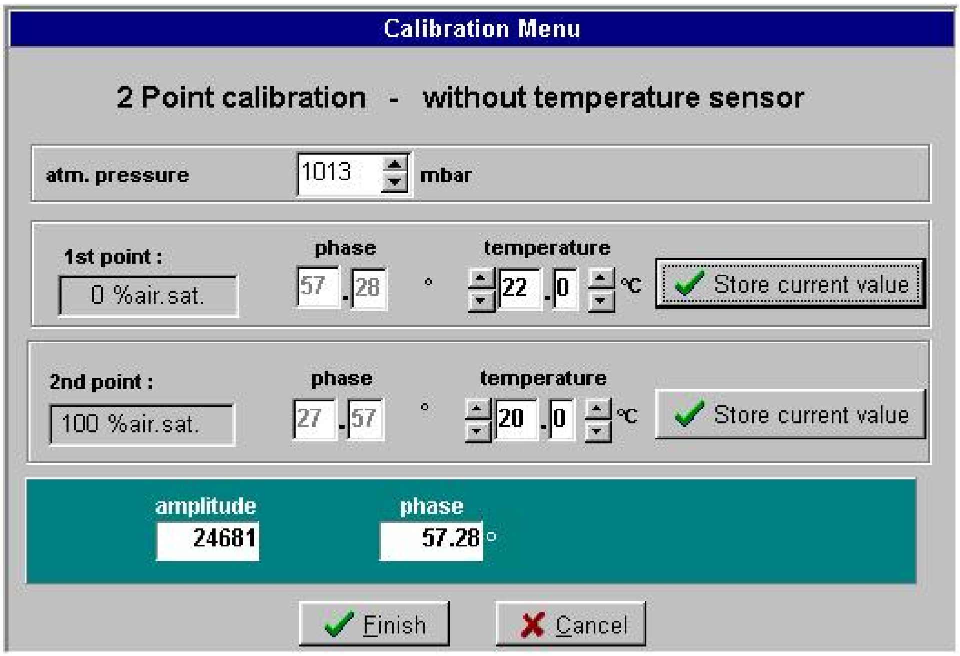
7. Wait about 3 minutes until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and click the ‘***Store current value***’ button to store the **100% air-sat.** value at the respective temperature.

   A message window opens and informs you that you will overwrite the existing calibration values. Click the ‘***Continue***‘ button to store the new calibration data.

8. To record the second calibration value, oxygen-free water (**cal 0**), remove the ***cal 100*** solution from the vessel and fill it with the calibration standard 0, **cal 0**. To minimize the response time, slightly stir the solution.
   Please note: Vigorous stirring can lead to oxygen contamination of the **cal 0** solution.

   Wait about 3 minutes until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the ‘***Store current value***’ button to store the **0% air-sat.** value at the respective temperature.

A message window opens and informs you that you will overwrite the existing calibration values. Click the ‘***Continue***‘ button to store the new calibration data.



12. Now calibration is complete. Confirm the calibration values by clicking the ‘***Finish***’ button.

13. Wash the planar sensor foil with distilled water to clean it from sodium sulfite. Remove the calibration solution 0 and fill the vial with distilled water. Stir the solution for about 1 minute. Repeat this washing procedure three times.

7.3.4 Calibration with Automatic Temperature Compensation

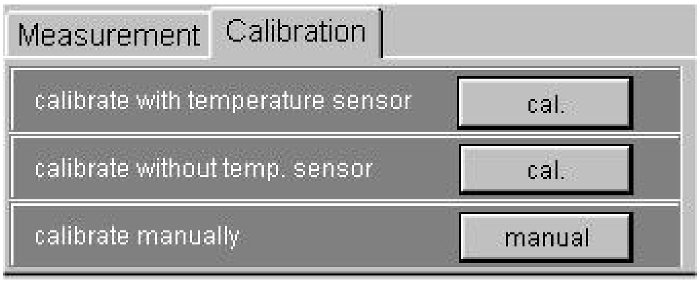
1. Connect the Fibox 3 via the RS232 cable to your computer.

2. To perform temperature-compensated measurements, connect the temperature sensor PT 1000 to the 4-pin connector on front of the Fibox 3. Fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the oxygen minisensor.

3. Switch on the Fibox 3 and connect the sensor as shown in Chapter 7.3.2 "***Mounting Planar Oxygen-Sensitive Foils***“.

4. Start the Fibox 3 software on your computer and click the **calibration** menu item.

5. Select the calibration routine ‘***calibrate with temperature sensor***‘ by clicking the respective ‘**cal.**’ button.



6. Enter the ‘***actual atmospheric pressure***’. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentration units (mg/L, µmol/L). Please note that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentration units (mg/L, µmol/L), but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.

7. Place the calibration solution 100 (**cal 100**), air-saturated water, into the glass vessel. To minimize the response time, slightly stir the solution.

   Insert the temperature sensor. Ensure that there is no temperature gradient between the oxygen sensor spot and the temperature sensor.

8. Wait about 3 minutes until the phase angle and the temperature value are constant (the variation of the phase angle and the temperature should be smaller than ± 0.05° and 0.1 °C, respectively) and press the ‘***Store current value***’ button to store both the ***100% air-sat.*** and ***temp at 100%*** values.

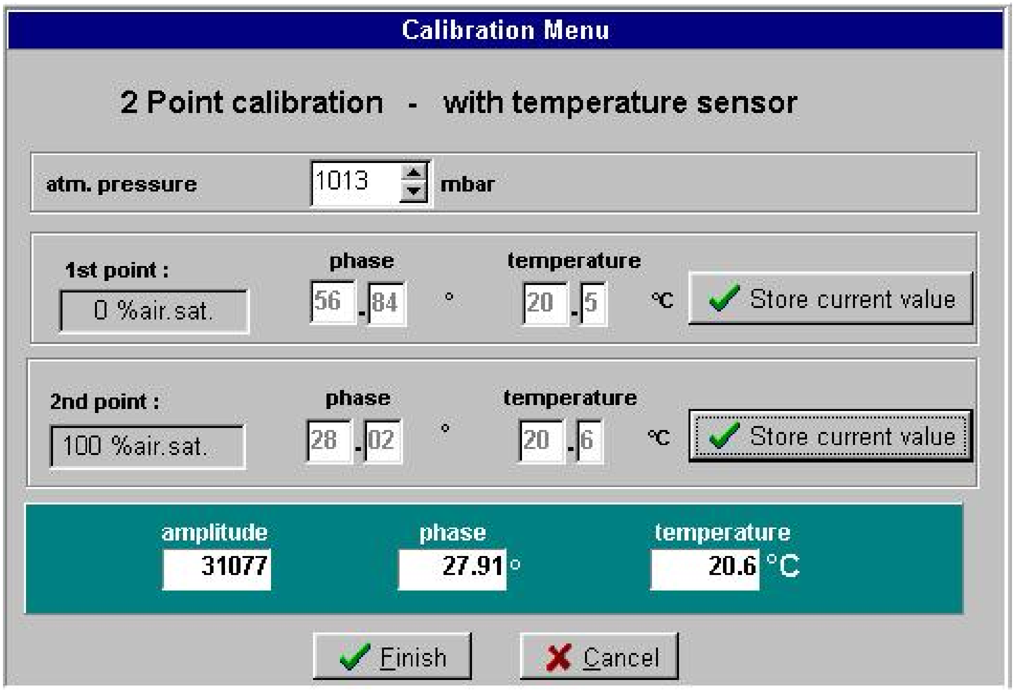
   A message window opens and informs you that you will overwrite the existing calibration values. Click the ‘***Continue***‘ button to store the new calibration data.

9. To record the second calibration value, oxygen-free water (**cal 0**), remove the ***cal 100*** solution from the vessel and fill it with the calibration standard 0, **cal 0**.

   Insert the temperature sensor into the glass vessel containing **cal 0**.

   To minimize the response time, slightly stir the solution.
   Please note that vigorous stirring can lead to oxygen contamination of the **cal 0** solution.

   Wait about 3 minutes until the phase angle and the temperature are constant (the variation of the phase angle and the temperature should be smaller than ± 0.05° and 0.1 °C, respectively) and click the ‘**Store *current value***’ button to store the **0% air-sat.** and **temp. at 0%** values.



A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

10. Now the calibration with temperature compensation is complete. Confirm the calibration values by clicking the '***Store'*** button.

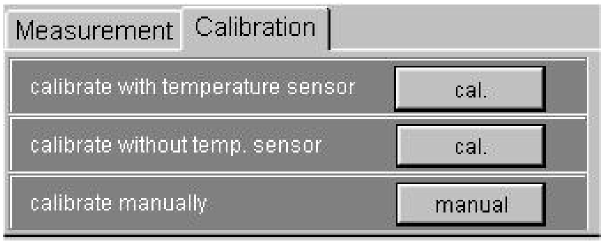
11. Wash the planar sensor foil with distilled water to clean it from sodium sulfite. Remove the **cal 0** and fill the vial with distilled water. Stir the solution for about 1 minute. Repeat this washing procedure three times.

    Wash also the temperature sensor with distilled water

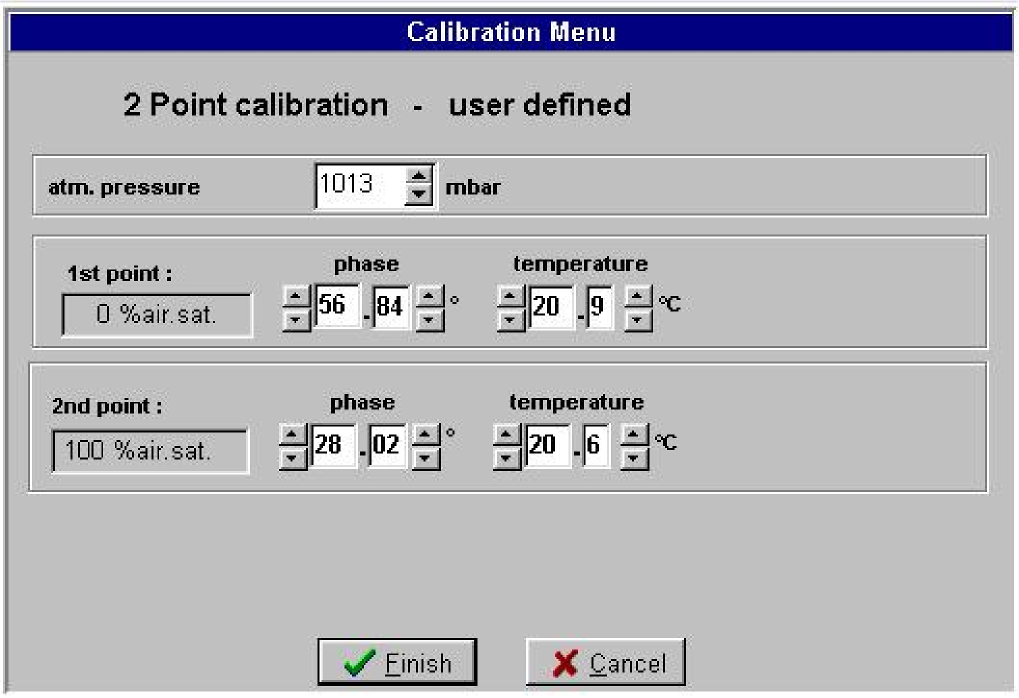
7.3.5 Manual Calibration

A manual calibration should be applied if you do not want to calibrate your sensor again. However, this is only possible if you already know the calibration values of the special sensor.

1. Connect the Fibox 3 via the RS232 cable to your computer.
2. Switch on the Fibox 3 oxygen meter.
3. Start the Fibox 3 software on your computer and click the **Calibration** menu item.
4. Select the calibration routine '**calibrate manually**' by clicking the **manual** button.



5. Enter the **atmospheric pressure** at which calibration was performed (**not** the **actual** one) and the respective calibration values **0 % air-sat., temp. at 0%** and **100 % air-sat., temp. at 100%**.



6. Now the user-defined calibration is complete. Confirm the calibration values by clicking the **Finish** button. A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

8 Measurement

Calibration of the sensor is recommended before each measurement (see chapter 7 *"Calibration*"). If you do not want to recalibrate a sensor, you can use the calibration values of your last measurement (see "*Manual Calibration*").

Each calibration is only valid for the corresponding sensor and should be repeated at least after every new start of the Fibox 3. Especially after longer measurements (more than 10000 measure points or 3 h continuous mode), the sensor should be re-calibrated.

Ensure that the temperature of the sample is known and is constant during measurement, if you do not use temperature compensation. In the case of temperature-compensated measurements, the temperature sensor PT 1000 should be positioned as close as possible to the oxygen minisensor to avoid temperature differences.

Please consult the special information on our Website www.presens.de or contact our service team if you are in any doubt.

8.1 Measurement with Dipping Probe (DP-PSt3)

1. Please carefully read chapter 7.1 "*Calibration of Oxygen Dipping Probe*"*.* There you will find relevant information about the proper handling of the oxygen-sensitive sensors. They are the basic for the following chapter.

2. Connect the Fibox 3 via the RS232 cable to your computer and switch on both.

3. For temperature-compensated measurement, connect the temperature sensor PT 1000 to the 4-pin connector on the front panel of the Fibox 3 and carefully tighten the safety nut. Fix the temperature sensor.

4. Calibrate the sensor according to chapter 7.1 "*Calibration of Oxygen Dipping Probe*. If you do not want to re-calibrate the sensor but use the calibration values of your last measurement, choose "*Manual".*

5. Position the oxygen-sensor in the sample. Ensure that no air bubbles are located at the sensor and that the temperature sensor is located close to the sensor in the case of temperature-compensated measurements.

8.2 Measurement with a Flow-Through Cell (FTC-PSt3)

1. Please carefully read chapter 7.2" *Calibration of Flow-Through Cell".* There you will find relevant information about the proper handling of oxygen flow-through sensors. They are the basic for the following chapter.

2. Connect the Fibox 3 via the RS232 cable to your computer.

3. Fix the male Luer-Lock adapter with the integrated 2 mm POF (polymer optical fiber) to the Luer T-connector and ensure that the fiber is located close to the glass tube.

4. Connect the temperature sensor PT 1000 to the 4-pin connector on the front panel of the Fibox 3 and carefully tighten the safety nut, to perform temperature-compensated measurement. Immerse the temperature sensor in your sample and fix it with a laboratory support.

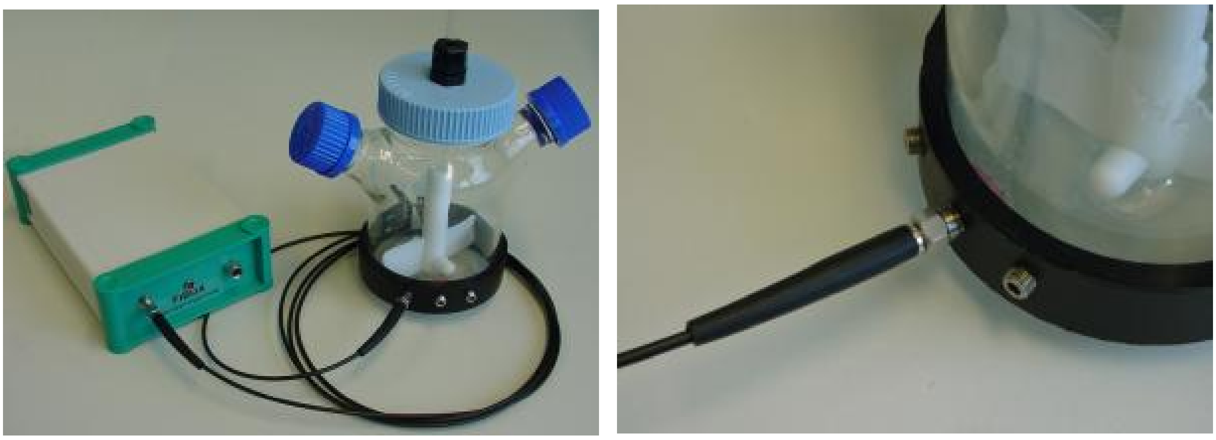
5. Calibrate the sensor according to chapter 7.2" ***Calibration of Flow-Through Cell*** ". If you do not want to re-calibrate the sensor but use the calibration values of your last measurement, choose "***Manual calibration***".

6. Connect the Luer-Lock adapter at the end-pieces of the T-connector with tubings and pump your sample through the flow-through cell.

   Ensure that no air bubbles are located in the flow-through cell.

8.3 Measurement with Oxygen-Sensitive Foils (SP-PSt3)

Integrated sensors are offered as single sensor spots of 25 mm^2 on a polyester or glass support or are already glued into sample flasks (e.g. cell culture flasks) containing a SMA holding device. Please contact our service team to find the optimal solution for your application.

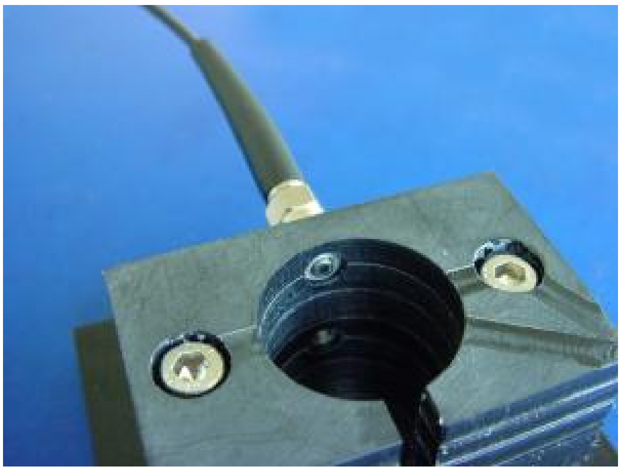


Cell culture flasks containing a holding device to connect SMA fiber bundles to read out the oxygen concentration through the flask wall.

1. Please carefully read chapter 7.3" ***Calibration of Planar Oxygen-Sensitive Foils***. There you will find relevant information about the proper handling of planar oxygen-sensitive foils integrated in glass vials. They are the basic for the following chapter.

2. Connect the Fibox 3 via the RS232 cable to your computer.

3. Remove the protective cap from the male fiber plugs of the delivered fiber cable and connect it to the SMA plugs of the Fibox 3 device and the holding device. The safety nut must be carefully attached while turning slightly clockwise.



4. For temperature-compensated measurement, connect the temperature sensor PT 1000 to the 4-pin connector on the front panel of the Fibox 3 and carefully tighten the safety nut. Immerse the temperature sensor into the glass vial with your sample and fix it with a laboratory support.

5. Calibrate the sensor according to chapter 7.3 "***Calibration of Planar Oxygen-Sensitive Foils***". If you have obtained sterile glass vials with an integrated oxygen sensor and hence are not able to calibrate the sensor, use the pre-calibration values of the inspection sheet you have obtained with the planar oxygen-sensitive foil. Choose "***Manual calibration***".

8.4 Some Advice for Correct Measurement

8.4.1 Signal drifts due to oxygen gradients

Please keep in mind that the sensor only measures the oxygen content near its surface. In unstirred solutions occurs often an oxygen concentration gradient.

Please check if air bubbles are on the sensor tip whenever unexpected drifts, gradients or unstable measurement values occur. Critical conditions for bubble formations are, for example, purging with air or other gases and increasing temperature during measurement.

The formation of a biofilm during long-term measurements or the accumulation of other sample components like oil or solid substances may lead to an oxygen gradient.

8.4.2 Signal drifts due to temperature gradients

A further source of imprecise measurement is insufficient temperature compensation. If you use the temperature compensation, ensure that no temperature gradients exist between the oxygen sensor and the temperature sensor. If you measure without temperature compensation, please bear in mind, that the Fibox 3 only measures correctly if the sample temperature is constant during measurement and the temperature is the same as you typed in at the beginning of the measurement. Please also refer to Chapter 13.5 "Formulas for temperature compensation". If the temperature is measured with a precision of ± 0.2 °C, there is a variation in the measuring value at 100% air-saturation of ± 0.7 % air-saturation. Please choose the measurement with temperature compensation to minimize temperature gradients.

8.4.3 Signal drift due to photodecomposition

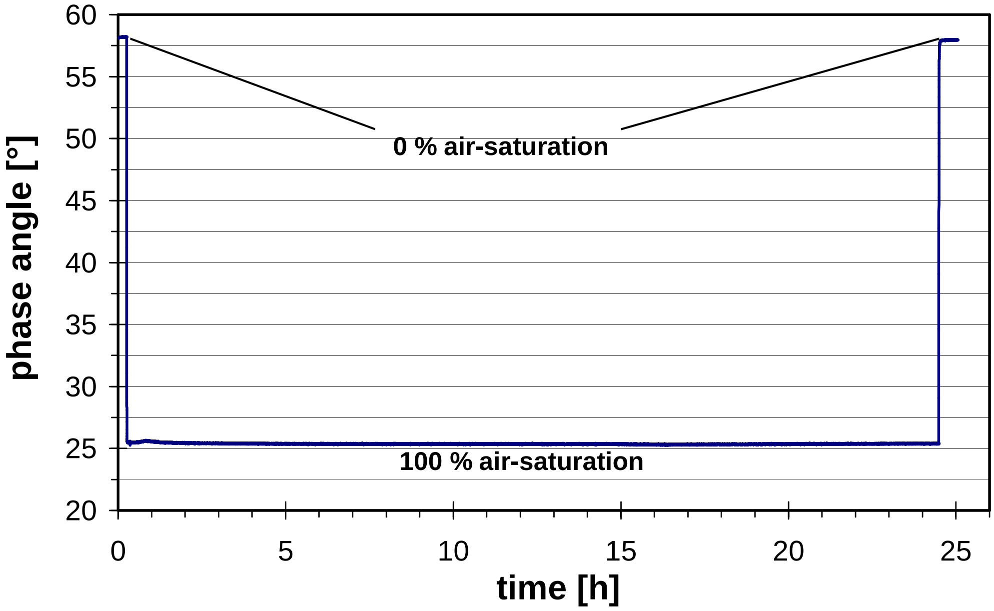
The oxygen-sensitive material may be subject to photodecomposition resulting in a signal drift. Photodecomposition takes place only during illumination of the sensor tip and depends on the intensity of the excitation light. Therefore, the excitation light was minimized.

Continuous illumination of a **DP-PSt3** oxygen sensor over a period of 24 hours may lead to a phase drift of up to + 0.4 % air-saturation measured at 100% air-saturation at 20°C. However, this effect of photodecomposition can even be minimized, by changing the measuring mode to the second or minute interval mode. In these modes, the software switches off the excitation light after recording the data point and switches it on after the interval you have chosen.

Please use the interval method whenever it is possible to increase the shelf life of the minisensor.

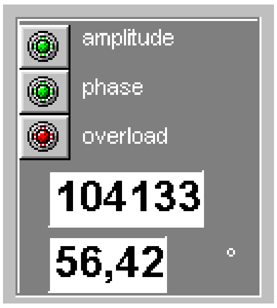
*Drift in % air-saturation at 100% air-saturation when illuminating the oxygen sensor **PSt3** for 1, 12 and 24 hours in the continuous mode.*

	Drift per hour	Drift per 12 hours	Drift per 24 hours
PSt3	---	---	< 0.4 % air-saturation



Photodecomposition test of **PSt3**, continuously illuminating the sensor membrane for 25 hours.

8.4.4 Signal drift due to too much ambient light



A source of error is the detector overload due to too much ambient light. A detector overload can be recognized with the red shining warning light ***overload***, which you can find at the right bottom of the window.

red: background light (e.g. direct sunlight, lamp) is too high. Decrease of false light by decreasing the light intensity or darkening the sample is necessary.

green: ratio of sensor signal to false light is acceptable

Please notify that your measurement is not reliable if the warning light overload is shining red. A detector overload causes a decrease in both amplitude and phase angle.

8.4.5 Performance proof

If you want to prove the performance during the past measurement, please check the calibration values by inserting the sensor tip in the '**cal 0**' and '**cal 100**' calibration standards when you have finished your measurement. If the device shows 0% air-saturation immersing the sensor tip into the '**cal 0**' solution and 100 % air-saturation measuring the '**cal 100**' standard, the sensor worked perfectly during the whole measurement.

8.4.6 Correction for air-pressure variations

The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentration units (mg/L µmol/L). The partial pressure and the oxygen concentration units are calculated from % air-saturation by the software. Consequently, changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentration units (mg/L, µmol/L), but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.

9 General Instructions

9.1 Warm-Up Time

The warm-up time of the electronic and opto-electronic components of the Fibox 3 is 5 min. Afterwards, stable measuring values are obtained.

9.2 Maintenance

The instrument is maintenance-free.

The housing should be cleaned only with a moist cloth. Avoid any moisture entering the housing! Never use benzine, acetone, alcohol or other organic solvents.

The SMA fiber connector of the minisensor can be cleaned only with lint-free cloth. The sensor tip may be rinsed only with distilled water or ethanol.

9.3 Service

Balancing, maintenance and repair work may only be carried out by the manufacturer:

PreSens
Precision Sensing GmbH
Josef-Engert-Straße 9
D-93053 Regensburg
Germany

Phone: +49 (0)941 942720
Fax: +49 941 9427227
E-mail: info@presens.de
Internet: www.presens.de

Please contact our service team should you have any questions. We look forward for helping you and are open for any questions and criticism.

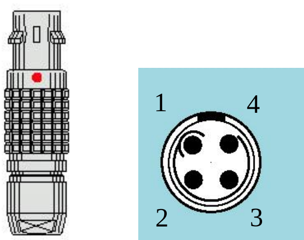
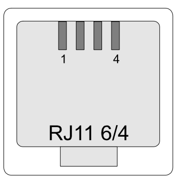
10 Technical Data

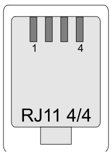
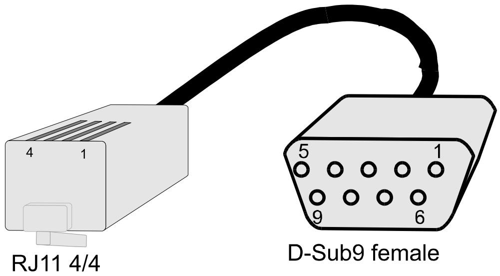
10.1 General Data

MODES	
PSt3 oxygen sensor	range: 0 - 250 % air-saturation resolution: 1 ± 0.05 % air-saturation 30 ± 0.1 % air-saturation 100 ± 0.5 % air-saturation 250 ± 1.7 % air-saturation accuracy: ± 1 % air-saturation at 100 % air-saturation ± 0.15% air-saturation at 1 % air-saturation
temperature	range: 0 - 50 °C resolution: ± 0.5 °C accuracy: ± 1° C

CALIBRATION PROCEDURE	
PSt3 oxygen sensor	2-point calibration in oxygen-free water and humidified air or air-saturated water

OPTICAL OUTPUT / INPUT	
Optical connector	SMA-compatible, 2 mm polymer optical fiber
Channels	1
Wavelength	505 nm

TEMPERATURE SENSOR INPUT	 1 ... PT1000-1 2 ... n.c. 3 ... n.c. 4 ... PT1000-2
Lemo Connector Size 00	Connector for PT 1000 temperature sensor
DC INPUT	DC-Range : 12 V/1250mA up to 18V/900mA  1 ... GND 2 ... +18VDC 3 ... GND 4 ... +18VDC

DIGITAL OUTPUT	
communication protocol	serial interface RS232 19200 Baud, Databits 8, Stoppbits 1, Parity none, Handshake none
instrument output:	on RJ11 4/4 plug  1 ... TXD 2 ... RXD 3 ... n.c. 4 ... GND
Interface cable to PC:	RJ11 4/4 to DSub9:  1 ... TXD 2 ... RXD 3 ... n.c. 4 ... GND 2 ... RXD 3 ... TXD 5 ... GND 1,4,6,7,8,9 ... n.c.

ENVIRONMENTAL CONDITIONS	
Operating temperature	0 to + 50ºC
Storage temperature	-10 to + 65ºC
Relative humidity:	up to 95%

OPERATION CONTROL	<u>*LED at the front panel:*</u> red: instrument off green: instrument on orange: stand by

DIMENSIONS	length: 185 mm width: 110 mm height: 45 mm weight: 630 g

10.2 Analog Output and External Trigger

The Fibox 3 instrument version is supplied with a dual programmable 12 bit analog output with galvanic isolation and an external trigger input.

- **ANALOG OUTPUT**

GENERAL SPECIFICATION - ANALOG OUTPUT

Channels	2
Connector	BNC
Resolution	12 bit
Output range	0 to 4095mV (±2mV max. error)
Galvanic isolation	500V rms
Shortcut protection	Yes

Programmable to oxygen, temperature, amplitude, phase by software

Equivalence coefficients :

oxygen	1 :: 0.1	(i.e. : 973 mV = 97.3 % air saturation)
temperature	1 :: 0.1	(i.e. : 208 mV = 20.8°C)
amplitude	1 :: 10	(i.e. : 2220 mV = 22200 relative units)
phase	1 :: 0.025	(i.e. : 1100 mV = 27.50°)

<u>Update rate:</u>
The update rate is dependent on the sampling rate of the software.
If an external trigger is used, the update rate is equivalent to the trigger pulse rate.

DC SPECIFICATION - ANALOG OUTPUT

Resolution

oxygen	± 2mV → ± 0.2 % air saturation
temperature	± 2mV → ± 0.2°C
amplitude	± 2mV → ± 20 relative units
phase	± 2mV → ± 0.05°)

Accuracy error ± 10mV

- **EXTERNAL TRIGGER INPUT**

GENERAL SPECIFICATION - EXTERNAL TRIGGER INPUT

Channels	1
Connector	BNC
Input voltage range	TTL-compatible / up to 24V
Trigger mode	Low-High-Low (Input must be kept Low for at least 50 µs)
Normal state	no current
Isolation	500 V rms

Timing Specifications:

Min rise &fall time for trigger	15 ns (see TTL-specification)
Max rise &fall time for trigger	2 ms
Min pulse length	3 ms
Min pause length	10 ms
Min period length	13 ms

10.3 Technical Notes

Power Adapter

Fibox 3 should always be used with the original power adapter (110-220VAC/12VDC). As an alternative power source a battery can be used that meets the DC input voltage given in technical specification. The battery adapter cable is available as an additional accessory.

Analog Outputs

WARNING: The analog outputs are not protected against any input voltage! Any voltage applied to the analog outputs can cause irreversible damage of the circuit.

RS232 Interface

The unit uses special interface cable. Another cable can cause the unit's malfunction.

Optical Output (ST)

The ST connector is a high precision optical component. Please keep it clean and dry. Always use the rubber cap to close the output when not in use.

10.4 Operation Notes

Oxygen Measurement

To achieve the highest accuracy Fibox 3 should be warmed-up for 5 min before starting the measurement. Please see the details of measurement process described in the Fibox 3 manual.

Temperature Compensation

No other than the supplied temperature sensor could be used with the unit. The use of any other temperature sensor can damage the oxygen meter.

11 Trouble Shooting

Error	Cause	Action
Device does not work and LED on the front panel is not lit	Device is not switched on No power supply	Switch on device with ON/OFF switch on the rear panel Connect power supply with device
Device does not work and LED on the front panel is on	No connection to PC	Check connection of the device to your PC (RS 232)
Temperature compensation failed, no temperature measurement possible	PT 1000 sensor is not connected PT 1000 sensor is faulty	Check connection Contact our service
Warning light: Amplitude: red	Oxygen sensor is not connected Sensor spot is removed from the plastic fiber (DP) Sensor spot (SP) is not illuminated sufficiently by polymer optical fiber Oxygen sensor is faulty	Check connection of the SMA connector Replace oxygen sensor (send the sensor back to PreSens for re-coating service) Check position of sensor spot and polymer optical fiber Replace oxygen sensor
Warning light: Phase: red	Phase angle out of limits	Check connection of the oxygen sensor Replace oxygen sensor
Warning light: Overload: red	Too much ambient light	Reduce ambient light by decreasing the light intensity Use optical-isolated oxygen sensors
No cal	Calibration failed Sensor was not in the right calibration standard Sulfite solution has aged	Calibrate again Check calibration solutions Immerse sensor in the proper calibration vessel Prepare new sulfite solution
Strong signal fluctuations	Air bubbles at sensor tip Too much ambient light Amplitude is too low	Remove air bubbles by carefully tapping Reduce ambient light by decreasing the light intensity Check amplitude

12 Concluding Remarks

Dear customer,
With this manual, we hope to provide you with an introduction to work with the Fibox 3 fiber-optic oxygen-meter.

This manual does not claim to be complete. We are endeavored to improve and supplement this version.
We are looking forward to your critical review and to any suggestions you may have.

You can find the newest version at www.presens.de

With best regards,

Your PreSens Team

13 Appendix

13.1 Basics in Optical Sensing of Oxygen

13.1.1 Dynamic Quenching of Luminescence

The principle of measurement is based on the effect of dynamic luminescence quenching by molecular oxygen. The following scheme explains the principle of dynamic luminescence quenching by oxygen.

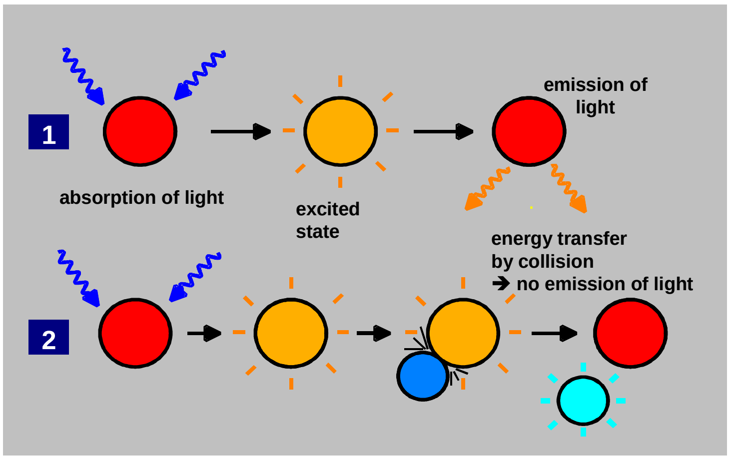


Figure 13.1 *Principle of dynamic quenching of luminescence by molecular oxygen*
(1) Luminescence process in absence of oxygen
(2) Deactivation of the luminescent indicator molecule by molecular oxygen

The collision between the luminophore in its excited state and the quencher (oxygen) results in radiationless deactivation and is called collisional or dynamic quenching. After collision, energy transfer takes place from the excited indicator molecule to oxygen which consequently is transferred from its ground state (triplet state) to its excited singlet state. As a result, the indicator molecule does not emit luminescence and the measurable luminescence signal decreases.

A relation exists between the oxygen concentration in the sample and the luminescence intensity as well as the luminescence lifetime which is described in the Stern-Volmer-equation (**1**). Here, τ_0 and τ are the luminescence decay times in absence and presence of oxygen (I_0 and I are the respective luminescence intensities), $[O_2]$ the oxygen concentration and K_{SV} the overall quenching constant.

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2]$$
$$I = f([O_2])$$
$$\tau = f([O_2]) \qquad (\mathbf{1})$$

I: Luminescence intensity in presence of oxygen
I_0: Luminescence intensity in absence of oxygen
τ: Luminescence decay time in presence of oxygen
τ_0: Luminescence decay time in absence of oxygen
K_{SV}: Stern-Volmer constant (quantifies the quenching efficiency and therefore the sensitivity of the sensor)
$[O_2]$: Oxygen content

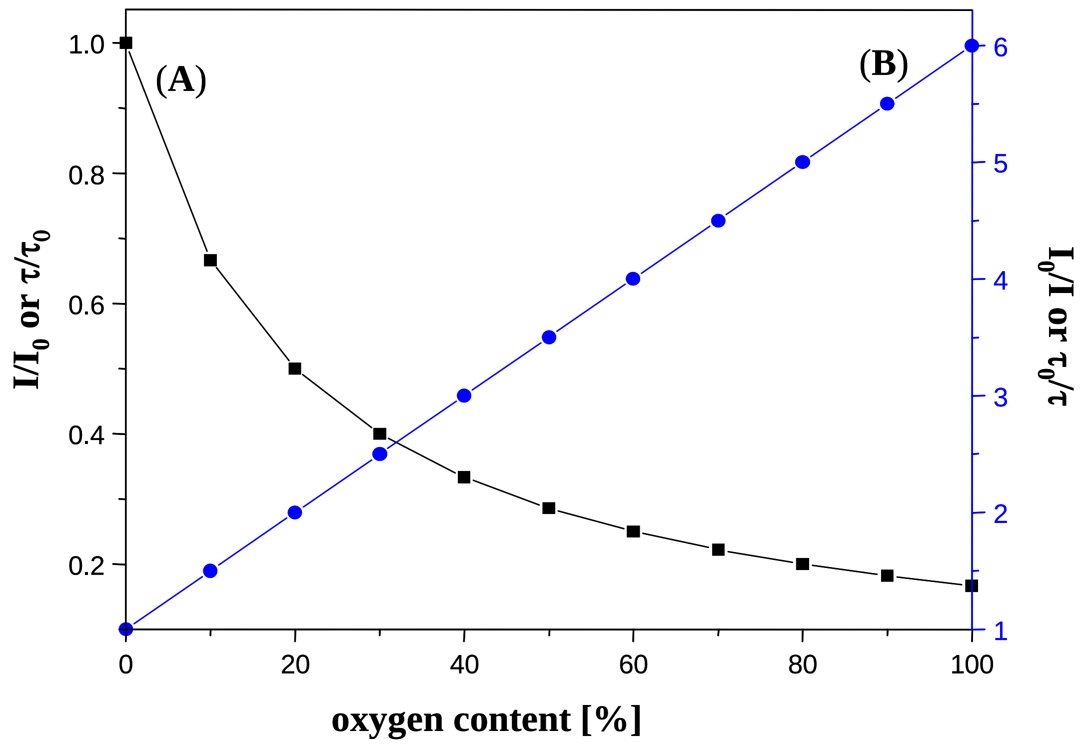


***Figure. 13.2** (**A**) Luminescence decrease in the presence of oxygen. (**B**) Stern-Volmer plot.*

Indicator dyes quenched by oxygen are, for example, polycyclic aromatic hydrocarbons, transition metal complexes of Ru(II), Os(II) and Rh(II), and phosphorescent porphyrins containing Pt(II) or Pd(II) as the central atom.

13.1.2 Major Components of Fiber-Optic Minisensors

In optical chemical sensors, the analyte interacts with an indicator and changes its optical properties. The result is either a change in the color (absorbance or spectral distribution) or the luminescence properties (intensity, lifetime, polarization). Light acts as the carrier of the information.

The major components of a typical fiber-optical sensing system are

- a light source to illuminate the sensor (laser, light emitting diode, lamps)
- an optical fiber as signal transducer (plastic or glass fiber)
- a photodetector (photodiode, photomultiplier tube, CCD-array)
- the optical sensor (indicator immobilized in a solid matrix)

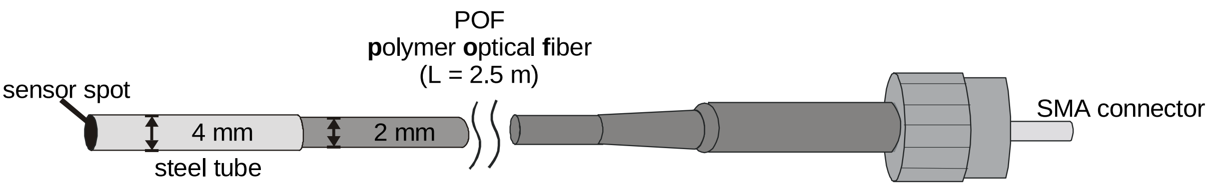


***Figure 13.3** Scheme of a minisensor.*

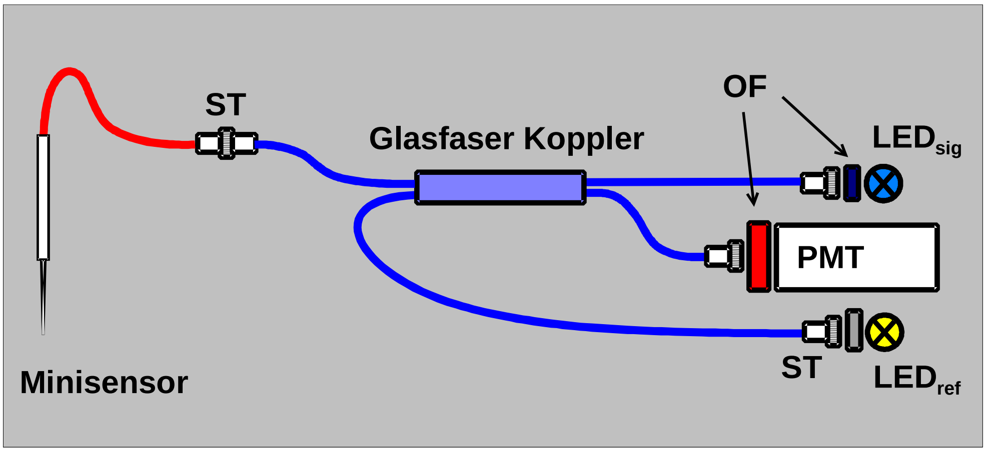


Figure 13.4 Schematic drawing of the optical setup of a measuring system with minisensors (LED: light emitting diodes, PMT: photomultiplier, OF: optical filters, ST: fiber connector).

13.1.3 Advantages of Optical Oxygen-Sensitive Minisensors

- no oxygen is consumed during the measurement;
- the signal is independent of changes in flow velocity;
- they are able to measure the oxygen content in dry gases
- they are insensible towards electrical interferences and magnetic fields;
- they are more sensitive than conventional electrodes (up to ppt-range);
- long-term stability and low drift;
- using silica fibers, it is possible to measure in samples while physically separate from the light source and detectors;
- light-conducting fibers are able to transport more information than power currents (information can be simultaneously transferred, e.g., intensity of light, spectral distribution, polarization, information such as decay time or delayed fluorescence);

13.1.4 Luminescence Decay Time

The Fibox 3 measures the luminescence decay time of the immobilized luminophore as the oxygen-dependent parameter.

$$\tau = f([O_2]) \qquad (\mathbf{2})$$

The Fibox 3 uses the phase modulation technique to evaluate the luminescence decay time of the indicators. If the luminophore is excited with light with sinusoidally modulated intensity, its decay time causes a time delay in the emitted light signal. In technical terms, this delay is the phase angle between the exciting and emitted signal. This phase angle is shifted as a function of the oxygen concentration. The relation between decay time τ and the phase angle Φ is shown by the following equation:

$$\tau = \frac{\tan\Phi}{2\pi \cdot f_{mod}} \qquad (\mathbf{3a})$$

$$\tan\Phi = 2\pi \cdot f_{mod} \cdot \tau \qquad (\mathbf{3b})$$

$$\tau \equiv \tan\Phi \equiv \Phi \equiv f([O_2]) \qquad (\mathbf{3c})$$

τ: luminescence decay time; Φ: phase angle; f_{mod}: modulation frequency

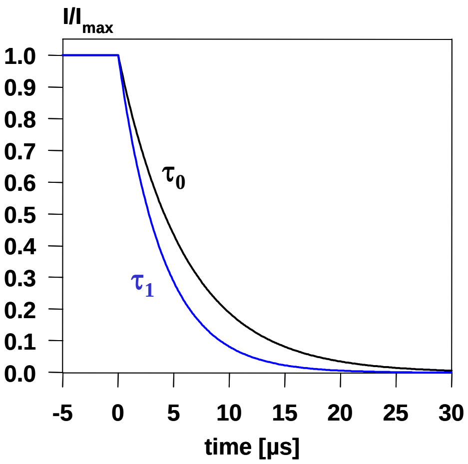


Figure 13.5 *Schematic of the single exponential decay ($t_0 > t_1$).*

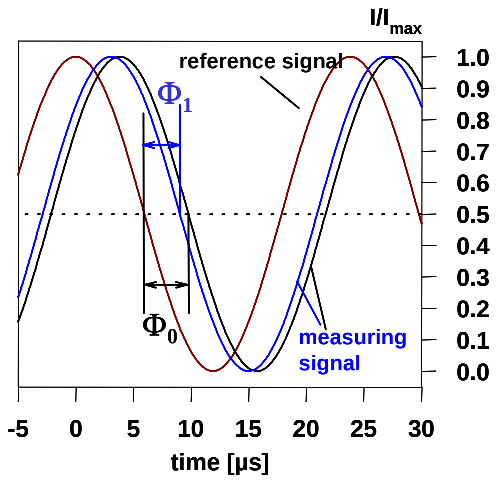


Figure 13.6 *The luminophore is excited with sinusoidally modulated light. Emission is delayed in phase expressed by the phase angle F relative to the excitation signal, caused by the decay time of the excited state*

The measurement of the luminescence decay time, an intrinsically referenced parameter, has the following advantages compared to the conventional intensity measurement:

- The decay time does not depend on fluctuations in the intensity of the light source and the sensitivity of the detector;
- The decay time is not influenced by signal loss caused by fiber bending or by intensity changes caused by changes in the geometry of the sensor;
- The decay time is, to a great extent, independent of the concentration of the indicator in the sensitive layer ➔ photobleaching and leaching of the indicator dye has no influence on the measuring signal;
- The decay time is not influenced by variations in the optical properties of the sample including turbidity, refractive index and coloration.

13.1.5 Literature

If you want to find out more about this subject, we recommend the following publications.

- Wolfbeis O.S. (Ed.), ***Fiber Optic Chemical Sensors and Biosensors***, Vol. **1**&**2**, CRC, Boca Raton (1991).
- Klimant I., Wolfbeis O.S., ***Oxygen-Sensitive Luminescent Materials Based on Silicone-Soluble Ruthenium Diimine Complexes***, Anal. Chem., **67**, 3160-3166 (1995).
- Klimant I., Kühl M., Glud R.N., Holst G., ***Optical measurement of oxygen and temperature in microscale: strategies and biological applications***, Sensors and Actuators B, **38-39**, 29-37 (1997).
- Holst G., Glud R.N., Kühl M., Klimant I., ***A microoptode array for fine-scale measurement of oxygen distribution***, Sensors and Actuators B, **38-39**, 122-129 (1997).
- Klimant I., Meyer V., Kühl M., ***Fiber-optic oxygen microsensors, a new tool in aquatic biology***, Limnol. Oceanogr., **40**, 1159-1165 (1995).
- Klimant I., Ruckruh F., Liebsch G., Stangelmayer A., Wolfbeis O.S., ***Fast Response Oxygen Microsensors Based on Novel Soluble Ormosil Glasses***, Mikrochim. Acta, **131**, 35-46 (1999).

13.2 Oxygen Conversion Formulas

Please note:

These conversion formulas are only valid in aqueous solutions and humidified air. These formulas have to be modified if measurements have to be performed in organic solvents or solutions with high salinity.

% saturation

% air-saturation

Default setting of the instrument.

% oxygen-saturation

$$\%\,O_2 = \%\ \text{air-saturation} \cdot \frac{20.95}{100} \qquad (4)$$

0.2095: volume content of oxygen in air

ppm in the gaseous phase:

$$\text{ppm}[O_2] = \%\ \text{air} - \text{saturation} \cdot \frac{20.95}{100} \cdot \frac{1}{10000} = \frac{\%\,O_2}{10000}$$

$$1\text{ppm} = \frac{1}{1000000} = \frac{1\text{mg}}{1\text{kg}} = \frac{1\mu\text{L}}{1\text{L}} = \frac{1}{10000}\% \qquad (5)$$

Partial pressure of oxygen

in hPa

$$p_{O_2}[\text{hPa}] = \left(p_{\text{atm}}[\text{hPa}] - p_W(T)[\text{hPa}]\right) \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \qquad (6)$$

in mbar

$$p_{O_2}[\text{mbar}] = \left(p_{\text{atm}}[\text{mbar}] - p_W(T)[\text{mbar}]\right) \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \qquad (7)$$

in Torr

$$p_{O_2}[\text{Torr}] = \left[\left(p_{\text{atm}}[\text{mbar}] - p_W(T)[\text{mbar}]\right) \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095\right] \cdot 0.75 \qquad (8)$$

Please note:

1 mbar = 1 hPa = 0.750 Torr

Oxygen Concentration

in mg/L

$$c_{O_2}[\text{mg/L}] = \frac{p_{\text{atm}} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M} \qquad (9)$$

in ppm = mg/L

$$c_{O_2}[ppm] = c_{O_2}[mg/L] = \frac{p_{atm} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M} \quad (10)$$

in μmol/L

$$c_{O_2}[\mu mol/L] = c_{O_2}[mg/L] \cdot \frac{1000}{M(O_2)} = c_{O_2}[mg/L] \cdot 31.25$$
$$= \frac{p_{atm} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air-saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000000 \cdot \frac{1}{V_M} \quad (11)$$

p_{atm}: actual atmospheric pressure

p_N: standard pressure (1013 mbar)

0.2095: volume content of oxygen in air

$p_W(T)$: vapor pressure of water at temperature T given in Kelvin

$\alpha(T)$: Bunsen absorption coefficient at temperature T; given in $cm^3(O_2)/cm^3$

$M(O_2)$: molecular mass of oxygen (32 g/mol)

V_M: molar volume (22.414 L/mol)

13.3 Temperature-Dependent Constants Affecting the Oxygen Content

13.3.1 Water Vapor Pressure

As shown in equation 4 - 11, the water vapor pressure p_w influences the oxygen partial pressure of air-saturated water and water vapor-saturated air.

Oxygen partial pressure in dry air:

$$p(O_2) = p_{atm} \cdot 0.2095 \quad (12)$$

$p(O_2)$: oxygen partial pressure in dry air at a barometric pressure p_{atm}
0.2095: volume content of oxygen in air.

Oxygen partial pressure in air-saturated water and water vapor-saturated air:

$$p(O_2)' = (p_{atm} - p_W(T)) \cdot 0.2095 \quad (13)$$

Temperature variations strongly affect water vapor pressure, and thus influence the oxygen partial pressure as shown in equation 13.

Table 13.1 Variation of water vapor pressure $p_W(T)$ with temperature.

θ [°C]	0	5	10	15	20	25	30	35	40	50
T [K]	273	278	283	288	293	298	303	308	131	323
$p_W(T)$ [mbar]	6.1	8.7	12.3	17.1	23.3	31.7	42.4	56.3	73.7	123.3

A convenient fitting function is given by the Campbell equation 14:

$$p_W(T) = \exp\left(A - \frac{B}{T} - C \cdot \ln T\right) \quad (14)$$

where T is the temperature in Kelvin and A, B and C constants given in Figure 13.7

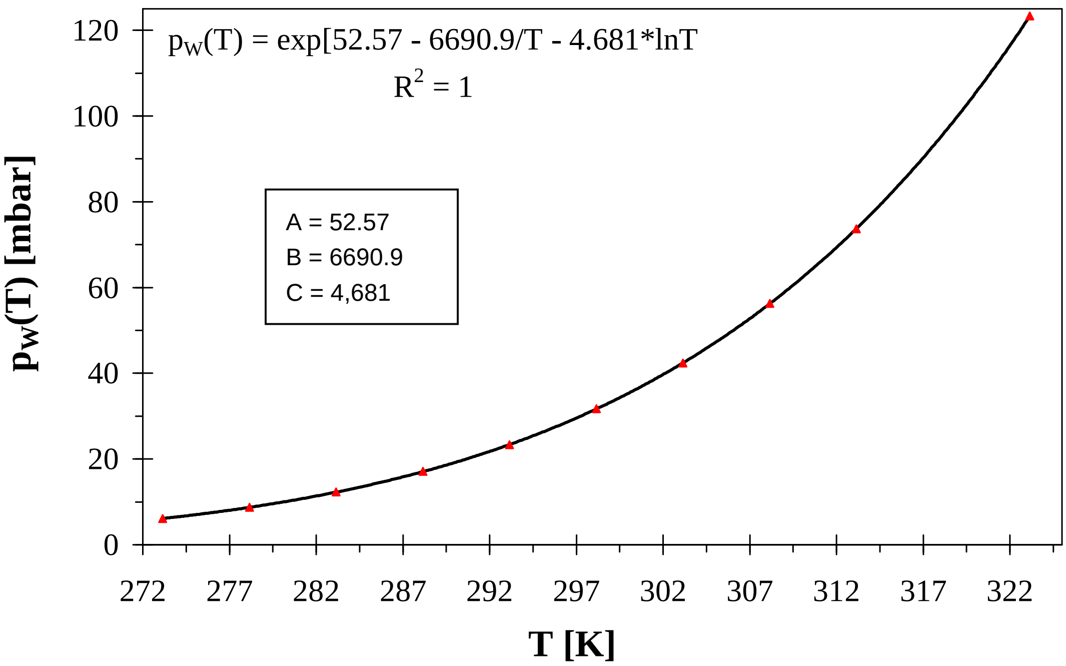


Figure. 13.7 *Variation of water vapor pressure with temperature. R^2 is the square of the correlation coefficient.*

13.3.2 Bunsen Absorption Coefficient

The solubility of oxygen in water is temperature-dependent and can be described using the Bunsen absorption coefficient $\alpha(\theta)$ and the oxygen partial pressure $p(O_2)$ according to equation 15. With increasing temperature, the solubility of oxygen in water decreases.

$$c_S(p,\theta) = \frac{p(O_2) - p_W(T)}{p_N}\alpha(\theta) \qquad (\mathbf{15})$$

$c_S(p,\theta)$: temperature-dependent solubility of oxygen in water, given in (cm^3 (O_2) / cm^3)

$p(O_2)$: oxygen partial pressure

p_N: standard pressure (1013 mbar)

$\alpha(\theta)$: Bunsen absorption coefficient, given in (cm^3 (O_2) / cm^3)

Table 13.2 Variation of Bunsen absorption coefficient $\alpha(\theta)$ with temperature.

θ [°C]	0	5	10	15	20	25	30	35	40	50
$\alpha(\theta)\cdot 10^3$	49.01	42.94	38.11	34.17	31.01	28.43	26.30	24.63	23.16	20.85

The data in Table 13.2 can be described by two forms of equations.

The first form of equation to describe the temperature-dependent variation of the Bunsen absorption coefficient $\alpha(\theta)$ is obtained by fitting a general power series to the values in Table 13.2. A fourth degree polynomial fit can be chosen, yielding equation 16.

$$10^3\alpha = a + b\cdot\theta + c\cdot\theta^2 + d\cdot\theta^3 + e\cdot\theta^4 \qquad (\mathbf{16})$$

where θ is the temperature in °C and a - e the coefficients calculated by standard curve fitting procedures as given in Figure 13.8.

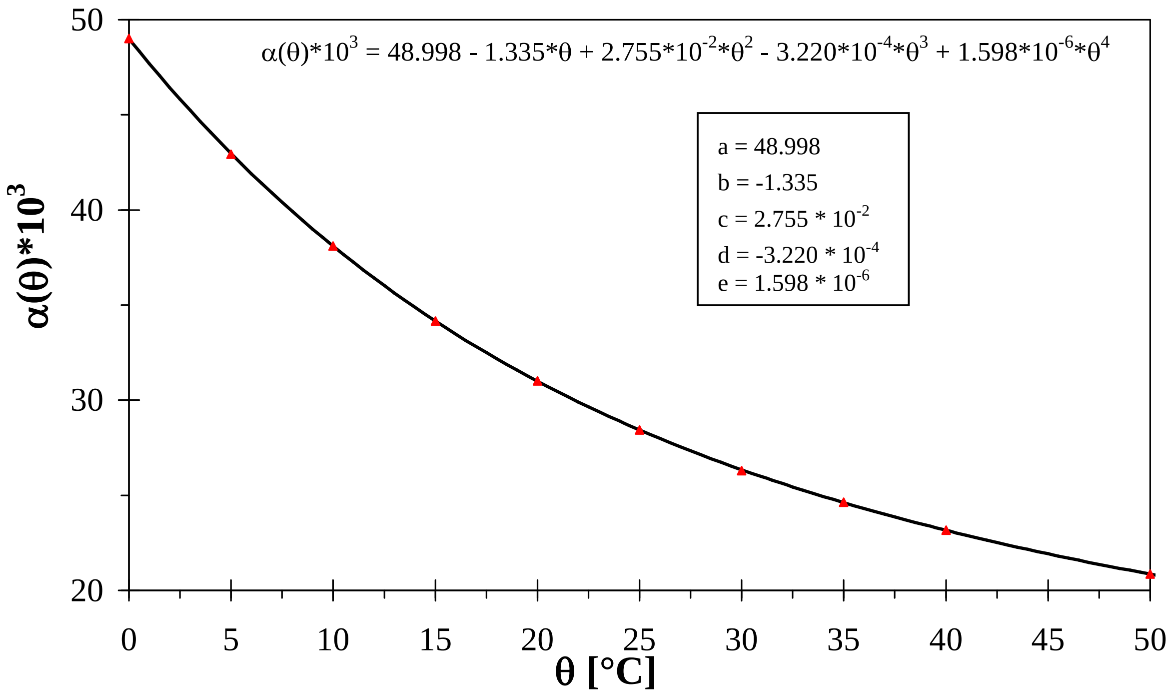


Figure 13.8 *Variation of Bunsen absorption coefficient $\alpha(\theta)$ with temperature. R^2 is the square of the correlation coefficient.*

The other form of equation to describe the variation of α with temperature can be derived from a thermodynamical correlation and gives an equation of the form

$$\ln 10^3 \alpha = \frac{A}{T} + B \cdot \ln T + C \tag{17}$$

where A, B and C are constants and T is the temperature in K. For oxygen dissolved in water we find by fitting the equation to the values of α in Table 13.2 that A = 8.553 * 10^3, B = 2.378 * 10, and C = -1.608 * 10^2.

Values of α calculated from eqns. 16 and 17 for the same temperature agree within ± 0.5 %.

The Bunsen absorption coefficient, however, is not a very practical measure. Values of $\alpha(\theta)$ have therefore to be converted to mg/L, and the method for doing this is best illustrated by an example.

Example: Calculation of the oxygen content ($c_S(p_{atm},\theta)$) in air-saturated water at a temperature θ of 20°C.

Equation 17 allows the solubility of oxygen in air-saturated fresh water to be calculated for any temperature and pressure provided that the values of the Bunsen absorption coefficient $\alpha(T)$ and the vapor pressure $p_W(T)$ at the particular temperature are known. Equation 16 or 17 can be used to obtain α, and p_W can be calculated from equation 14. The oxygen content c_S of air-saturated water can be calculated according to

$$c_S(p_{atm},\theta) = \frac{p_{atm} - p_W(\theta)}{p_N} \cdot 0.2095 \cdot \alpha(\theta) \cdot \frac{M_{O_2}}{V_M}. \tag{18}$$

In equation 18, p_{atm} is the actual atmospheric pressure corrected for the contribution of the water vapor pressure p_w and related to standard pressure p_N. The corrected pressure is multiplied by 0.2095, the volume content of oxygen in air, by $\alpha(\theta)$ and by the molecular mass of oxygen (M_{O2}) divided by the molar volume V_M.

At a given atmospheric pressure of 1013 mbar ($p_{atm} = p_N$) and a temperature of 20 °C the oxygen content can be calculated according to equation 19 and results in

$$c_S(1013\,\mathrm{mbar}, 20°\mathrm{C}) = \frac{1013 - 23.3}{1013} \cdot 0.2095 \cdot 0.031 \cdot \frac{32 \cdot \mathrm{g/mol}}{22.414\,\mathrm{mol/L}} = 0.009\,\mathrm{g/L} = 9.06\,\mathrm{mg/L} \qquad (19)$$

Table 13.3 gives oxygen solubilities in mg/L for temperature intervals of 0.1 °C from 0-40°C. The calculated value for c_s at a temperature of 20.0 °C agrees with the tabulated value of 9.08 mg/L.

Figure 13.9 shows the temperature-dependent oxygen solubility in air-saturated fresh water.

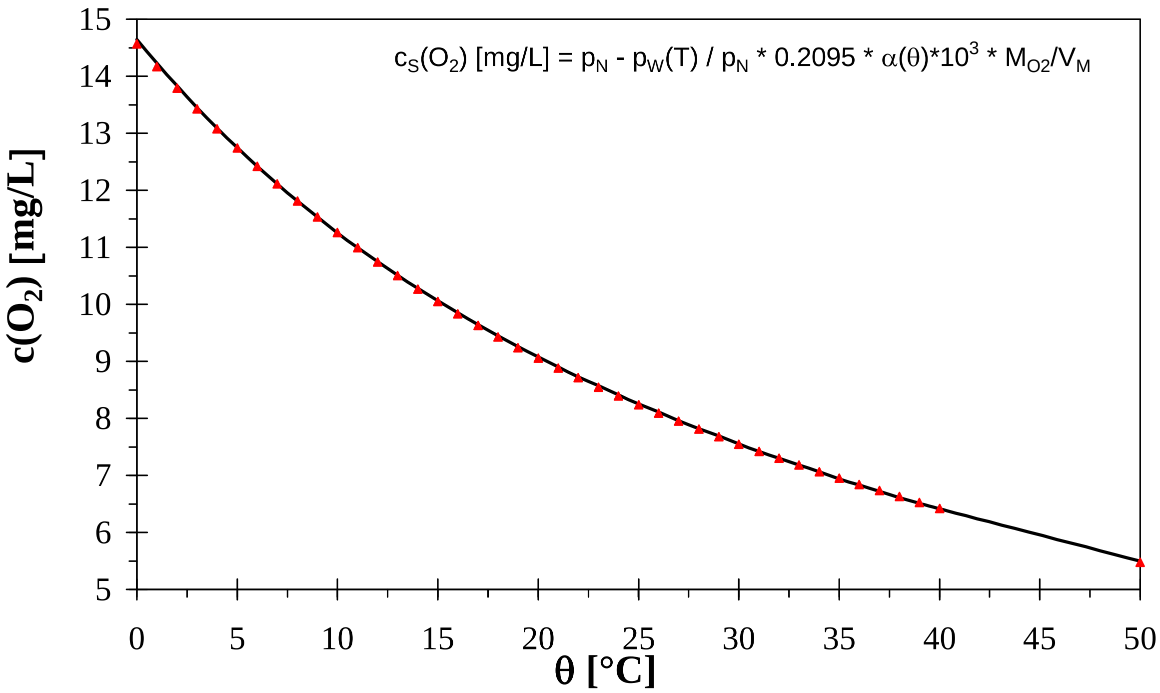


Figure 13.9 *Dependence of the oxygen solubility in air-saturated fresh water on temperature.*

Table 13.3 Oxygen solubility in air-saturated fresh water [mg/L].

T [°C]	c_S(T)	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0
0	14.	64	60	55	51	47	43	39	35	31	27	23
1		23	19	15	10	06	03	99	95	91	87	83
2	13.	83	79	75	71	68	64	60	56	52	49	45
3		45	41	38	34	30	27	23	20	16	12	09
4		09	05	02	98	95	92	88	85	81	78	75
5	12.	75	71	68	65	61	58	55	52	48	45	42
6		42	39	36	32	29	26	23	20	17	14	11
7		11	08	05	02	99	96	93	90	87	84	81
8	11.	81	78	75	72	69	67	64	61	58	55	53
9		53	50	47	44	42	39	36	33	31	28	25
10		25	23	20	18	15	12	10	07	05	02	99
11	10.	99	97	94	92	89	87	84	82	79	77	75
12		75	72	70	67	65	63	60	58	55	53	51
13		51	48	46	44	41	39	37	35	32	30	28
14		28	26	23	21	19	17	15	12	10	08	06
15		06	04	02	99	97	95	93	91	89	87	85
16	9.	85	83	81	70	76	74	72	70	68	66	64
17		64	62	60	58	56	54	53	51	49	47	45
18		45	43	41	39	37	35	33	31	30	28	26
19		26	24	22	20	19	17	15	13	11	09	08
20		08	06	04	02	01	99	97	95	94	92	90
21	8.	90	88	87	85	83	82	80	78	76	75	73
22		73	71	70	68	66	65	63	62	60	58	57
23		57	55	53	52	50	49	47	46	44	42	41
24		41	39	38	36	35	33	32	30	28	27	25
25		25	24	22	21	19	18	16	15	14	12	11
26		11	09	08	06	05	03	02	00	99	98	96
27	7.	96	95	93	92	90	89	88	86	85	83	82
28		82	81	79	78	77	75	74	73	71	70	69
29		69	67	66	65	63	62	61	59	58	57	55
30		55	54	53	51	50	49	48	46	45	44	42
31		42	41	40	39	37	36	35	34	32	31	30
32		30	29	28	26	25	24	23	21	20	19	18
33		18	17	15	14	13	12	11	09	08	07	06
34		06	05	04	02	01	00	99	98	97	96	94
35	6.	94	93	92	91	90	89	88	87	85	84	83
36		83	82	81	80	79	78	77	75	74	73	72
37		72	71	70	69	68	67	66	65	64	63	61
38		61	60	59	58	57	56	55	54	53	52	51
39		51	50	49	48	47	46	45	44	43	42	41
40		41	40	39	38	37	36	35	34	33	32	31

Example:: c_S(**20.0°C**) = **9.08 mg/L**

13.3.3 Dependence on the Salt Concentration

Table 13.4 gives values of the concentration of dissolved oxygen at several temperatures in solutions with various chloride concentrations. Increasing the salt concentration leads to a decrease in oxygen solubility. This behavior is characteristic for the solubility of many nonelectrolytes - it is the phenomenon known as the ***salting-out effect***.

Instead of chlorinity [Cl^-] - the amount of chloride in parts per thousand - which was used as a measure of the amount of salt in water, the term salinity is often used. If salinity is preferred as a measure of salt concentration, then the conversion from g/L can be readily made using equation 20.

$S = 1.805[Cl^-] + 0.03$ (**20**)

where S is the salinity in [‰] or [g/1000g].

Table 13.4 Solubility of oxygen in water as a function of temperature and salt concentration (Total pressure = 760 torr)

T [°C]	***Oxygen solubility [mg/L]***					
[Cl⁻] (g/1000g)	**0**	**4**	**8**	**12**	**16**	**20**
0	14.5	13.9	13.3	12.6	12.0	11.3
10	11.3	10.8	10.4	9.9	9.5	9.0
20	9.1	8.8	8.5	8.1	7.8	7.4
30	7.5	7.3	7.0	6.7	6.4	6.1

The effect of increasing the salt concentration on the vapor pressure is negligible small as shown in Table 13.5.

Table 13.5. Variation of solution vapor pressure (p_W) with salt concentration

T [°C]	***Vapor pressure of solution (torr)***			
[Cl⁻] (g/1000g)	**0**	**9**	**18**	**26**
0	4.6	4.5	4.4	4.4
10	9.2	9.1	8.9	8.8
20	17.5	17.3	17.0	16.7
30	31.8	31.4	30.9	30.4

The dependence of oxygen solubility on salt concentration can also be obtained from equation 14 except that now values calculated from either equation 21 or 22 have to be used for calculation of the Bunsen absorption coefficient. Equation 21 differs from equation 16 by an additional forth degree polynomial term for chlorinity.

$$10^3 \cdot \alpha = a + b\cdot\theta + c\cdot\theta^2 + d\cdot\theta^3 + e\cdot\theta^4 - [Cl^-]\cdot(p + q\cdot\theta + r\cdot\theta^2 + s\cdot\theta^3 + t\cdot\theta^4) \qquad (\mathbf{21})$$

where θ is the temperature in °C, a - e are the coefficients used in equation 16 and p - t are new constants given in Table 13.6. The values of these new constants are obtained by fitting the polynomial to experimental data in the ranges $0 \le \theta \le 30$°C and $0 \le [Cl^-] \le 20$‰. To obtain an oxygen solubility from the Bunsen absorption coefficient, the same procedure as described previously is used (s. equation 18, page 74).

An alternative equation to compensate the Bunsen absorption coefficient by the salt concentration displays equation 22.

$$10^3 \cdot \alpha = \exp\left[\left(A + \frac{B}{T} + C\cdot\ln T + D\cdot T\right) - [Cl^-]\cdot\left(P + \frac{Q}{T} + R\cdot\ln T + S\cdot T\right)\right] \qquad (\mathbf{22})$$

where T is the temperature in Kelvin, and A - D and P - S are the coefficients given in Table 13.6. They are based on measurements for $273.1 \le T \le 308.18$ K and $0 \le [Cl^-] \le 30$‰ and is therefore more extensive than equation 21. Both equations give values of $10^3 \cdot \alpha$ which agree to better than ± 1 %.

Table 13.6 Values of the coefficients in equations **21** and **22**.

Eqn. 21	a	$4.900 * 10$	p	$5.516 * 10^{-1}$
	b	-1.335	q	$-1.759 * 10^{-2}$
	c	$2.759 * 10^{-2}$	r	$2.253 * 10^{-4}$
	d	$-3.235 * 10^{-4}$	s	$-2.654 * 10^{-7}$
	e	$1.614 * 10^{-2}$	t	$5.362 * 10^{-8}$
Eqn. 22	A	-7.424	P	$-1.288 * 10^{-1}$
	B	$4.417 * 10^{3}$	Q	$5.344 * 10$
	C	-2.927	R	$-4.442 * 10^{-2}$
	D	$4.238 * 10^{-2}$	S	$7.145 * 10^{-4}$

Seawater has a typical salinity of 35 ‰ (35 g / 1000 g) or a chloride content of about 19 ‰, and therefore falls within the scope of both equations.